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(54) Title: INTERFERON TAU COMPOSITIONS AND METHODS OF USE

| Peptides | MW | HI* | Sequence |
|---|---|---|---|
| IFNt(1-37) (SEQ ID NO:5) | 4465 | -0.78 | CYSLRKLMLDARENLKLLDRMNRLSPHSCLQDRKDFG |
| IFNt(34-64) (SEQ ID NO:6) | 3610 | -0.72 | KDFGLPQEMVEGDQLQKDQAFPVLYEMLQQS |
| IFNt(62-92) (SEQ ID NO:7) | 3586 | -0.53 | QQSFNLFYTEHSSAAWDTTLLEQLCTGLQQQ |
| IFNt(90-122) (SEQ ID NO:8) | 3712 | -0.86 | QQQLDHLDTCRGQVMGEEDSELGNMDPIVTVKK |
| IFNt(119-150) (SEQ ID NO:9) | 3948 | -0.56 | TVKKYFQGIYDYLQEKGYSDCAWEIVRVEMMR |
| IFNt(139-172) (SEQ ID NO:10) | 3818 | -0.11 | CAWEIVRVEMMRALTVSTTLQKRLTKMGGDLNSP |

*Hydropathic Index

(57) Abstract

The present invention describes the production of interferon-τ proteins and polypeptides derived therefrom. The antiviral and anticellular proliferation properties of these proteins and polypeptides are disclosed. One advantage of the proteins of the present invention is that they do not have cytotoxic side-effects when used to treat cells. Structure/function relationships for the interferon-τ protein are also described.

# INTERFERON TAU COMPOSITIONS AND METHODS OF USE

## Field of the Invention

The present invention relates to interferon-τ compositions and methods of use.

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## Background of the Invention

Conceptus membranes, or trophectoderm, of various mammals produce biochemical signals that allow for the establishment and maintenance of pregnancy (Bazer, *et al.*, 1983). One such protein, ovine trophoblast protein-one (oTP-1), was identified as a low molecular weight protein secreted by sheep conceptuses between days 10 and 21 of pregnancy (Wilson, *et al.*, 1979; Bazer, *et al.*, 1986). The protein oTP-1 was shown to inhibit uterine secretion of prostaglandin $F_2$-alpha, which causes the corpus luteum on the ovary to undergo physiological and endocrinological demise in nonpregnant sheep (Bazer, *et al.*, 1986). Accordingly, oTP-1 has antiluteolytic bio-

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logical activity.  The primary role of oTP-1 was assumed
to be associated with the establishment of pregnancy.

oTP-1 was subsequently found to (i) exhibit limited
homology (50-70%) with interferon alphas (IFNα) of vari-

5  ous species (Imakawa, et al., 1987), and (ii) bind to a
Type I interferon receptor (Stewart, et al., 1987).
Despite some similarities with IFNα, oTP-1 has several
features that distinguish it from IFNα including the
following:  oTP-1's role in reproductive biochemistry

10  (other interferons are not known to have any role in the
biochemical regulation of reproductive cycles), oTP-1's
cellular source -- trophoblast cells (IFNα is derived
from lymphocytes cells), oTP-1's size -- 172 amino acids
(IFNα is typically about 166 amino acids), and oTP-1 is

15  weakly inducible by viruses (IFNα is highly inducible by
viruses).  The International Interferon Society  recog-
nizes oTP-1 as belonging to an entirely new class of
interferons which have been named interferon-tau (IFNτ).
The Greek letter τ stands for trophoblast.

20      The interferons have been classified into two dis-
tinct groups: type I interferons, including IFNα, IFNβ,
and IFNω (also known as IFNαII); and type II interferons,
represented by IFNγ (reviewed by DeMaeyer, et al.).  In
humans, it is estimated that there are at least 17 IFNα

25  non-allelic genes, at least about 2 or 3 IFNβ non-allelic
genes, and a single IFNγ gene.

IFNα's has been shown to inhibit various types of
cellular proliferation.  IFNα's are especially useful
against hematologic malignancies such as hairy-cell

30  leukemia (Quesada, et al., 1984).  Further, these pro-
teins have also shown activity against multiple myeloma,
chronic lymphocytic leukemia, low-grade lymphoma,
Kaposi's sarcoma, chronic myelogenous leukemia, renal-
cell carcinoma, urinary bladder tumors and ovarian can-

cers (Bonnem, *et al.*, 1984; Oldham, 1985). The role of interferons and interferon receptors in the pathogenesis of certain autoimmune and inflammatory diseases has also been investigated (Benoit, *et al.*, 1993).

5      IFNα's are also useful against various types of viral infections (Finter, *et al.*, 1991). Alpha interferons have shown activity against human papillomavirus infection, Hepatitis B, and Hepatitis C infections (Finter, *et al.*, 1991; Kashima, *et al.*, 1988; Dusheiko,

10   *et al.*, 1986; Davis, *et al.*, 1989). Significantly, however, the usefulness of IFNα's has been limited by their toxicity: use of interferons in the treatment of cancer and viral disease has resulted in serious side effects, such as fever, chills, anorexia, weight loss,

15   and fatigue (Pontzer, *et al.*, 1991; Oldham, 1985). These side effects often require (i) the interferon dosage to be reduced to levels that limit the effectiveness of treatment, or (ii) the removal of the patient from treatment. Such toxicity has reduced the usefulness of these

20   potent antiviral and antiproliferative proteins in the treatment of debilitating human and animal diseases.

**Summary of the Invention**
      In one aspect the present invention includes a

25   method of inhibiting tumor cell growth. In the method, cells are contacted with interferon-τ (IFNτ) at a concentration effective to inhibit growth of the tumor cells. IFNτ can be obtained from a number of sources including cows, sheep, and humans. Two embodiments include the

30   IFNτ presented as either SEQ ID NO:2 or SEQ ID NO:4. A number of tumor cells can be targeted for growth inhibition by IFNτ, including but not limited to the following: human carcinoma cells and steroid-affected tumor cells (*e.g.*, mammary tumor cells).

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The present invention also includes a method of
inhibiting viral replication in a cell.  In this method
cells infected with a virus are contacted with interfer-
on-τ at a concentration effective to inhibit viral repli-
5    cation within the cells.  The IFNτ molecules described
above are also useful in this method of the present
invention.  The replication of a number of viruses can be
inhibited in cells, these viruses include RNA (e.g.,
feline leukemia virus, human immunodeficiency virus, or
10   Hepatitis C Virus) and DNA (e.g., Hepatitis B Virus)
viruses.

The human IFNτ molecules of the present invention
can also be used in a method of enhancing fertility in a
female mammal.  In this method an amount of human IFNτ
15   effective to enhance fertility of a female mammal is
administered, typically in a pharmaceutically acceptable
carrier.  Exemplary of such human IFNτ molecules are the
protein sequences presented as SEQ ID NO:4 and SEQ ID
NO:12.

20   The present invention also includes an isolated
nucleic acid which encodes a human interferon-τ.  Exem-
plary of such nucleic acid molecules are SEQ ID NO:3 and
SEQ ID NO:11.  Further, the invention includes expression
vectors for the expression of human IFNτ.  Typically the
25   expression vector includes (a) a nucleic acid containing
an open reading frame that encodes human interferon-τ;
and (b) regulatory sequences effective to express said
open reading frame in a host cell.  The regulatory se-
quence may include sequences useful for targeting or
30   secretion of the IFNτ polypeptide:  such sequences may be
endogenous (such as the normally occurring IFNτ leader
sequences, see SEQ ID NO:11) or heterologous (such as a
secretory signal recognized in yeast or bacterial expres-
sion systems).  In the expression vector, regulatory
35   sequences may also include, 5' to said nucleic acid

sequence, a promoter region and an ATG start codon in-
frame with the interferon-τ coding sequence, and 3' to
said coding sequence, a translation termination signal
followed by a transcription termination signal.  The
5    nucleic acid in the expression vector may be selected
from, for example, SEQ ID NO:1, SEQ ID NO:3 and SEQ ID
NO:11.

In another embodiment, the present invention in-
cludes a recombinantly produced human interferon-τ pro-
10    tein.  One exemplary sequence for such a protein is given
as SEQ ID NO:4.  Human IFNτ may also contain a carboxy
terminal extension (such as the sequence presented as SEQ
ID NO:12).

The invention includes a method of recombinantly
15    producing interferon-τ.  In the method, a recombinant
expression system containing an open reading frame (ORF)
having a polynucleotide sequence which encodes a human
interferon-τ polypeptide, where the vector is designed to
express the ORF in the host, is introduced into suitable
20    host cells.  The host is then cultured under conditions
resulting in the expression of the ORF sequence.  The
human IFNτ sequences discussed above are examples of
suitable human IFNτ polypeptides.  Examples of polynucle-
otide coding sequences are SEQ ID NO:3 and SEQ ID NO:11.
25    Numerous vectors and their corresponding hosts are useful
in the practice of this method of the invention, includ-
ing, lambda gt11 phage vector and *E. coli* cells.  Other
host cells include, yeast and insect cell expression
systems.

30    The invention further includes expression systems
useful for the expression of interferon-τ polypeptides.
Typically these systems include a host capable of sup-
porting expression of an open reading frame in a selected
expression vector, and the selected expression vector
35    containing an open reading frame (ORF) having a polynu-

cleotide sequence which encodes a human interferon-τ
polypeptide.  Exemplary of sequences that can be utilized
in such expression systems are SEQ ID NO:4, SEQ ID NO:15,
SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19
5     and SEQ ID NO:20.

The invention also includes isolated interferon-τ
polypeptides.  These polypeptides are derived from the
interferon-τ amino acid coding sequence and are between
about 15 and 172 amino acids in length.  Such polypep-
10    tides can be selected, for example, from the sequences
presented as SEQ ID NO:2 and SEQ ID NO:4.  Exemplary
IFNτ-derived polypeptides include, but are not limited
to, the following:  SEQ ID NO:5, SEQ ID NO:7, SEQ ID
NO:10, SEQ ID NO:15, SEQ ID NO:17, and SEQ ID NO:20.

15    In another embodiment, the invention includes a
method of blocking the binding of alpha-interferon to a
cell having an alpha-interferon receptor.  In this method
the cell is contacted with an interferon-τ polypeptide at
a concentration effective to allow the binding of inter-
20    feron-τ to each alpha-interferon receptor.  The cells,
having the IFNτ polypeptide bound to the receptor, are
then exposed to alpha-interferon (IFNα).  The IFNτ poly-
peptides and IFNτ-derived polypeptides described above
are examples of IFNτ polypeptides useful in this method.

25    Further, the invention includes a method of blocking
the binding of interferon-τ to a cell having a interfer-
on-τ receptor.  In this method, the cell is contacted
with an interferon-τ-derived polypeptide (e.g., SEQ ID
NO:5, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:15, SEQ ID
30    NO:17 or SEQ ID NO:20), where the polypeptide is at a
concentration effective to allow the binding of the
polypeptide to each interferon-τ-receptor.  The cells are
then exposed to interferon-τ.

The invention also includes purified antibodies that
35    are immunoreactive with human interferon-τ.  The antibod-

ies may be polyclonal or monoclonal.  Exemplary IFNτ
polypeptide antigens include, but are not limited, to the
following:  SEQ ID NO:4, SEQ ID NO:15, SEQ ID NO:17 and
SEQ ID NO:20.

5      The present invention also includes the following:
interferon-τ-derived polypeptides that have anti-tumor
(i.e., anti-proliferative) activity; interferon-τ-derived
polypeptides that have anti-viral activity; and hybrid α-
interferon molecules in which the toxicity portion of
10   native IFNα has been replaced by analogous sequences from
IFNτ.

These and other objects and features of the inven-
tion will be more fully appreciated when the following
detailed description of the invention is read in conjunc-
15   tion with the accompanying drawings.


**Brief Description of the Figures**

Figure 1 presents the nucleic acid coding sequence
20   of a synthetic gene of OvIFNτ designed to include 19
unique restriction enzyme sites spaced evenly throughout
the coding sequence.

Figure 2 shows the cloning strategy used for making
a synthetic gene encoding OvIFNτ.

25     Figure 3 shows a comparison of the predicted protein
sequences of a human interferon-τ gene and an ovine
interferon-τ gene.  Divergent amino acids are indicated
by presentation of the alternative amino acid on the line
below the nucleic acid sequences.

30     Figure 4 presents data demonstrating that both
OvIFNτ and IFNα were able to drastically reduce growth of
HL-60 cells.

Figure 5 presents data demonstrating that rHuIFNα is
cytotoxic and OvIFNτ is not.  In the figure, results of

one of three replicate experiments are presented as mean
% viability ± SD.

Figure 6 presents the sequences of polypeptides
derived from the IFNτ sequence.

5         Figure 7 presents the complete nucleic acid and
amino acid sequence of an OvIFNτ sequence.

Figure 8 presents data supporting the lack of cyto-
toxicity, relative to IFNα, when IFNτ is used to treat
peripheral blood mononuclear cells.

10         Figure 9 shows the results of treatment of a human
cutaneous T cell lymphoma line, HUT 78, with IFNτ.

Figure 10 shows the results of treatment of a human
T cell lymphoma line, H9, with IFNτ.

Figure 11A presents data for the peptide inhibition,
15    relative to FIV (feline immunodeficiency virus) replica-
tion, of polypeptides derived from OvIFNτ with whole
OvIFNτ.  Figure 11B presents data for the peptide inhibi-
tion, relative to HIV (human immunodeficiency virus)
replication, of polypeptides derived from OvIFNτ with
20    whole OvIFNτ.

Figure 12 presents data demonstrating the inhibition
of the antiviral activity of IFNτ by IFNτ-derived pep-
tides.

Figure 13 presents data demonstrating the inhibition
25    by IFNτ-derived peptides of OvIFNτ antiviral activity.

Figure 14 presents data demonstrating the inhibition
by IFNτ-derived peptides of bovine IFNα antiviral activi-
ty.

Figure 15 presents data demonstrating the inhibition
30    by IFNτ-derived peptides of human IFNα antiviral activi-
ty.

Figure 16 presents data evaluating the lack of
inhibition by IFNτ-derived peptides of bovine IFNγ anti-
viral activity.

Figure 17 presents data demonstrating the anti-IFNτ-derived peptide antisera inhibition of the antiviral activity of IFNτ.

Figure 18 presents data demonstrating the anti-IFNτ-derived peptide antisera inhibition of the binding of radiolabeled IFNτ to cells.

**Brief Description of the Sequences**

SEQ ID NO:1 is the nucleotide sequence of a synthetic gene encoding ovine interferon-τ (OvIFNτ). Also shown is the encoded amino acid sequence.

SEQ ID NO:2 is an amino acid sequence of a mature OvIFNτ protein.

SEQ ID NO:3 is a synthetic nucleotide sequence encoding a mature human interferon-τ (HuIFNτ) protein.

SEQ ID NO:4 is an amino acid sequence for a mature HuIFNτ protein.

SEQ ID NO:5 is the amino acid sequence of fragment 1-37 of SEQ ID NO:2.

SEQ ID NO:6 is the amino acid sequence of fragment 34-64 of SEQ ID NO:2.

SEQ ID NO:7 is the amino acid sequence of fragment 62-92 of SEQ ID NO:2.

SEQ ID NO:8 is the amino acid sequence of fragment 90-122 of SEQ ID NO:2.

SEQ ID NO:9 is the amino acid sequence of fragment 119-150 of SEQ ID NO:2.

SEQ ID NO:10 is the amino acid sequence of fragment 139-172 of SEQ ID NO:2.

SEQ ID NO:11 is the nucleotide sequence of a natural HuIFNτ gene with a leader sequence.

SEQ ID NO:12 is the predicted amino acid coding sequence of the SEQ ID NO:11.

SEQ ID NO:13 is a 25-mer synthetic oligonucleotide according to the subject invention.

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SEQ ID NO:14 is a 25-mer synthetic oligonucleotide according the subject invention.

SEQ ID NO:15 is the amino acid sequence of fragment 1-37 of SEQ ID NO:4.

5      SEQ ID NO:16 is the amino acid sequence of fragment 34-64 of SEQ ID NO:4.

SEQ ID NO:17 is the amino acid sequence of fragment 62-92 of SEQ ID NO:4.

SEQ ID NO:18 is the amino acid sequence of fragment
10    90-122 of SEQ ID NO:4.

SEQ ID NO:19 is the amino acid sequence of fragment 119-150 of SEQ ID NO:4.

SEQ ID NO:20 is the amino acid sequence of fragment 139-172 of SEQ ID NO:4.

15

**Detailed Description of the Invention**

I.   Definitions.

*Interferon-τ* refers to any one of a family of interferon proteins having the following characteristics:   (i)
20    anti-luteolytic properties; (ii) anti-viral properties; (iii) anticellular proliferation properties; (iv) 45-68% amino acid homology with α-Interferons and greater than 70% amino acid homology to the sequence presented as SEQ ID NO:2.   Interferon-τ can be isolated from a number of
25    mammalian sources as described below.

An *interferon-τ polypeptide* is a polypeptide having between about 15 and 172 amino acids derived from an interferon-τ amino acid coding sequence, where said 15 to 172 amino acids are contiguous in native interferon-τ.
30    Such 15-172 amino acid regions can also be assembled into polypeptides where two or more such interferon-τ regions are joined that are normally discontinuous in the native protein.

II.  Isolation and Characterization of Interferon-τ.
A.  Ovine and Bovine Interferon-τ.
1.  Interferon-τ Coding Sequences.

Ovine interferon-τ (OvIFNτ) is a major conceptus
secretory protein produced by the embryonic trophectoderm
during the critical period of maternal recognition in
sheep.  One isolate of mature OvIFNτ is 172 amino acids
in length (SEQ ID NO:2).  The cDNA coding sequence con-
tains an additional 23 amino acids at the amino-terminal
end of the mature protein (Imakawa, et al., 1987).  The
coding sequence of this OvIFNτ isolate is presented as
Figure 7.

For the isolation of OvIFNτ protein, conceptuses
were collected from pregnant sheep and cultured in vitro
in a modified Minimum Essential Medium as described
previously (Godkin, et al., 1982).  Conceptuses were
collected on various days of pregnancy with the first day
of mating being described as Day 0.  IFNτ was purified
from conceptus culture medium essentially as described by
Vallet, et al., (1987) and Godkin, et al. (1982).

The homogeneity of IFNτ was assessed by sodium
dodecyl sulfate polyacrylamide gel electrophoresis (SDS-
PAGE; Maniatis, et al.; Ausubel, et al.).  Determination
of protein concentration in purified IFNτ samples was
performed using the bicinchoninic (BCA) assay (Pierce
Chemical Co., Rockford, IL; Smith, et al., 1985).

A homologous protein to OvIFNτ was isolated from
cows (bIFNτ; Helmer, et al., 1987; Imakawa, et al.,
1989).  OvIFNτ and BoIFNτ (i) have similar functions in
maternal recognition of pregnancy, and (ii) share a high
degree of amino acid and nucleotide sequence homology
between mature proteins.  The nucleic acid sequence
homology between OvIFNτ and BoIFNτ is 76.3% for the 5'
non-coding region, 89.7% for the coding region, and 91.9%

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for the 3' non-coding region.  The amino acid sequence homology is 80.4%.

Example 1 describes the reproductive functions of OvIFNτ.  OvIFNτ and recombinant human α-2-Interferon
5      (rHuIFNα₂) were infused into uterine lumen of ewes at a variety of concentrations.  The life span of the corpus luteum was assessed by examination of interestrous intervals, maintenance of progesterone secretion, and inhibition of prostaglandin secretion (Davis, et al., 1992).
10     Comparison of the data resulting from these examinations demonstrated a considerable lengthening of the interestrous interval when IFNτ is administered at 100μg/day and no meaningful effect when rHuIFNα is administered. These data support the conclusion that IFNτ significantly
15     influences the biochemical events of the estrous cycle.

The antiviral properties of interferon-τ at various stages of the reproductive cycle were also examined (Example 2).  Conceptus cultures were established using conceptus obtained from sheep at days 12 through 16 of
20     the estrus cycle.  Antiviral activity of supernatant from each conceptus culture was assessed.  Culture supernatants had increasing antiviral activity associated with advancing development of the conceptus up to Day 16 post estrus.
25

2.  Recombinant Production of IFNτ.

Recombinant IFNτ was produced using bacterial and yeast cells.  The amino acid coding sequence for OvIFNτ was used to generate a corresponding DNA coding sequence
30     with codon usage optimized for expression in E. coli (Example 3).  The DNA coding sequence was synthetically constructed by sequential addition of oligonucleotides. Cloned oligonucleotides were fused into a single polynucleotide using the restriction digestions and ligations

outlined in Figure 2. The polynucleotide coding sequence had the sequence presented as SEQ ID NO:1.

For expression of recombinant IFNτ, this synthetic coding sequence can be placed in a number of bacterial

5     expression vectors: for example, lambda gt11 (Promega, Madison WI); pGEX (Smith, *et al.*); pNH vectors (Strata-gene, La Jolla CA). Cloning of the IFNτ synthetic poly-nucleotide into a modified pIN III omp-A expression vector is described in Example 3. Production of the IFNτ

10    protein was induced by the addition of IPTG. Soluble recombinant IFNτ was liberated from the cells by sonica-tion or osmotic fractionation.

The protein can be further purified by standard methods, including size fractionation (column chromatog-

15    raphy or preoperative gel electrophoresis) or affinity chromatography (using, for example, anti-IFNτ antibodies (solid support available from Pharmacia, Piscataway NJ). Protein preparations can also be concentrated by, for example, filtration (Amicon, Danvers, Mass.).

20    The synthetic IFNτ gene was also cloned into the yeast cloning vector pBS24Ub (Example 4; Sabin, *et al.*; Ecker, *et al.*). Synthetic linkers were constructed to permit in-frame fusion of the IFNτ coding sequences with the ubiquitin coding sequences in the vector. The re-

25    sulting junction allowed *in vivo* cleavage of the ubiqui-tin sequences from the IFNτ sequences.

The recombinant plasmid pBS24Ub-IFNτ was transformed into the yeast *S. cerevisiae*. Transformed yeast cells were cultured, lysed and the recombinant IFNτ (r-IFNτ)

30    protein isolated from the cell lysates.

The amount of r-IFNτ was quantified by radioimmuno-assay. Microsequencing of the purified r-IFNτ was car-ried out. The results demonstrated identity with native IFNτ through the first 15 amino acids. The results also

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confirmed that the ubiquitin/IFNτ fusion protein was
correctly processed *in vivo.*

Recombinant IFNτ obtained by this method exhibited
antiviral activity similar to the antiviral activity of
5     IFNτ purified from conceptus-conditioned culture medium.

Other yeast vectors can be used in the practice of
the present invention including, but are not limited to,
vectors with regulatable expression (Hitzeman, *et al.;*
Rutter, *et al.;* Oeda, *et al.*). The yeast transformation
10    host is typically *Saccharomyces cerevisiae,* however,
other yeast suitable for transformation can be used as    .
well (*e.g., Schizosaccharomyces pombe*).

The DNA encoding the IFNτ polypeptide can be cloned
into any number of commercially available vectors to
15    generate expression of the polypeptide in the appropriate
host system.  These systems include the above described
bacterial and yeast expression systems as well as the
following:  baculovirus expression (Reilly, *et al.;*
Beames, *et al.;* Clontech, Palo Alto CA); and expression
20    in mammalian cells (Clontech, Palo Alto CA; Gibco-BRL,
Gaithersburg MD).  These recombinant polypeptides can be
expressed as fusion proteins or as native proteins.  A
number of features can be engineered into the expression
vectors, such as leader sequences which promote the
25    secretion of the expressed sequences into culture medium.
The recombinantly produced polypeptides are typically
isolated from lysed cells or culture media.  Purification
can be carried out by methods known in the art including
salt fractionation, ion exchange chromatography, and
30    affinity chromatography.  Immunoaffinity chromatography
can be employed, as described above, using antibodies
generated based on the IFNτ polypeptides.

B.    Human Interferon-τ.

1.    Identification and Cloning of Human Genomic
      Sequences Encoding an Interferon-τ Protein.

DNA was screened for sequences homologous to inter-
5    feron-τ (Example 5). Several sequences that hybridized
with the OvIFNτ cDNA probe were identified. Several
clones containing partial sequences of human interferon-τ
were then isolated (Example 6). Two synthetic 25-mer
oligonucleotides, corresponding to sequences from the
10   OvIFNτ cDNA (Imakawa, et al., 1987) were synthesized.
These primers were employed in amplification reactions
using DNA derived from the following two cDNA libraries:
human term placenta and human term cytotrophoblast. The
resulting amplified DNA fragments were electrophoreti-
15   cally separated and a band containing an IFNτ amplifica-
tion product was isolated. The product was subcloned and
the inserted amplification product sequenced using the
dideoxy termination method.

Comparison of sequences from three of the clones
20   revealed a high degree of sequence homology between the
isolates, but the sequences were not identical. This
result suggests the existence of multiple variants of
human interferon-τ genes.

Example 7 describes the isolation of a full-length
25   human IFNτ gene. High molecular weight DNA was isolated
from peripheral blood mononuclear cells (PBMCs) and size-
fractionated. Fractions were tested for the presence of
IFNτ sequences using polymerase chain reaction: DNA
molecules from fractions that tested amplification posi-
30   tive were used to generate a subgenomic library in λgt11.

This subgenomic library was plated and hybridized
with an OvIFNτ cDNA probe (Example 7A). Approximately 20
clones were identified that hybridized to the probe.
Plaques corresponding to the positive clones were pas-
35   saged, DNA isolated and analyzed by amplification reac-

20

tions using OvIFNτ primers.  Of these twenty plaques, six
plaques generated positive PCR signals.  The phage from
these six clones were purified and the inserts sequenced.
One of the inserts from one of these six clones was used
5      as a hybridization probe in the following screening.
        Recombinant phage from the λgt11 subgenomic library
were screened using the hybridization probe just de-
scribed (Example 7B).  Three clones giving positive
hybridization signals were isolated and the inserts
10     sequenced.  The resulting nucleic acid sequence informa-
tion is presented as SEQ ID NO:11 and the predicted
protein coding sequence is presented as SEQ ID NO:12.
The predicted mature protein coding sequence is presented
as SEQ ID NO:4.
15          Comparison of the predicted protein sequences (Fig-
ure 3) of the human interferon-τ gene (SEQ ID NO:4) and
the ovine interferon-τ gene demonstrates the levels of
sequence homology and divergence at the amino acid level.
        The human IFNτ sequences presented as SEQ ID NO:12
20     and SEQ ID NO:11, and primers and probes derived there-
from, can be used as specific probes to detect isolates
of further human IFNτ coding sequences and/or pseudo-
genes.  Further, there may be more than one isoform of
the IFNτ protein and more than one coding sequence per
25     species.  The specific nucleic acid probes used in the
practice of the present invention and antibodies reactive
with the IFNτ polypeptides of the present invention may
be useful to isolate unidentified variants of interferon-
τ in mammals, according to the methods of the invention
30     disclosed herein.

2.    <u>Characterization of the Expression of Interfe-
      ron-τ in Human Tissues.</u>

Human placental cDNA libraries and an ovine cDNA
library were analyzed by hybridization to the OvIFNτ cDNA
5    probe (Example 8).  This DNA hybridization analysis sug-
gested that the IFNτ-signals from human cDNA libraries
were approximately 1/100 of the signal obtained using the
ovine cDNA library.  OvIFNτ cDNAs constitute around 0.4%
of the ovine cDNA library.  Accordingly, the abundance of
10   human cDNAs responding to the OvIFNτ probe appears to be
low, at least in the term placenta from which the cDNA
libraries were generated.

The presence of HuIFNτ mRNA in human term placenta
and amniocytes were also analyzed.  The results suggest
15   the presence of human IFNτ mRNA in the feto-placental
annex.  The aminocytes also expressed the messages corre-
sponding to OvIFNτ primers and human probe, suggesting
that the expression of IFNτ mRNA is not limited to the
term placenta.

20   In addition, a RT-PCR analysis for the presence of
HuIFNτ was applied to the total cellular RNA isolated
from human adult lymphocytes:  the results suggest that
IFNτ mRNA exists in lymphocytes.

The expression of interferon-τ in human tissue was
25   also examined using *in situ* hybridization (Example 9).
Sections from four healthy, different term and first tri-
mester human placentas were examined.  This analysis em-
ployed a cDNA probe derived from the OvIFNτ cDNA sequen-
ces (Example 9B).  *In situ* hybridization was performed
30   using an anti-sense RNA probe.  In three separate experi-
ments, specific hybridization was observed in all term
and first trimester placental tissues.

First trimester placental villi (composed of an
outer layer of syncytiotrophoblast, an underlying layer
35   of cytotrophoblast, and a central stromal region with

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various types of mesenchymal cells) displayed the highest
transcript level of IFNτ in the cytotrophoblast cells.
Less intense but detectable levels were present in both
the syncytiotrophoblast and stromal cells.  A similar
5    pattern of transcript expression was demonstrated in the
placental villi of term tissue but the level of signal
detection was low.  First trimester extravillous tropho-
blasts displayed the highest amount of message and
stained positive when present in the maternal blood
10   spaces.

Howatson, et al., (1988) noted IFNα production in
the syncytiotrophoblast of chorionic villi in both first
trimester and term tissues.  Also, Paulesu, et al. (1991)
observed IFNα in extravillous trophoblast as well as
15   syncytiotrophoblasts, noting more intense and abundant
reactivity in first trimester placental tissue when
compared to those taken at term.  These investigators
employed antibodies raised against human IFNα subtypes,
and none observed any IFNα in the villous cytotropho-
20   blasts.

The present results demonstrate that the human IFNτ
gene is highly expressed in early placental tissues by
migrating extravillous trophoblasts, but is also ex-
pressed in villous syncytiotrophoblasts, cytotropho-
25   blasts, and various stromal cells.  These results demon-
strate the detection of IFNτ transcripts in human preg-
nancy tissues, and IFNτ expression in the villous cyto-
trophoblasts as well as the extravillous trophoblast of
first trimester placenta.

30

C.    Antiviral Properties of Interferon-τ.
The antiviral activity of IFNτ has been evaluated
against a number of viruses, including both RNA and DNA
viruses.  The relative specific activity of OvIFNτ,
35   purified to homogeneity, was evaluated in antiviral

assays (Example 10). IFNτ had a higher specific antiviral activity than either rBoIFNα or rBoIFNγ (Example 10, Table 3).

One advantage of the present invention is that IFNτ has potent antiviral activity with limited cytotoxic effects. Highly purified OvIFNτ was tested for antiretroviral and cytotoxic effects on peripheral blood lymphocytes exposed to feline AIDS and human AIDS retroviruses (Bazer, F.W., et al., (1989)). This feline AIDS lentivirus produces a chronic AIDS-like syndrome in cats and is a model for human AIDS (Pederson, et al., 1987). Replication of either virus in peripheral blood lymphocytes (PBL) was monitored by reverse transcriptase (RT) activity in culture supernatants over time.

To determine IFNτ antiviral activity against FIV and HIV, RNA-dependent DNA polymerase RT activity was assayed in FIV- and HIV-infected feline and human PBL cultures treated with IFNτ (Example 11). Replication of FIV was reduced to about one-third of control values when cells were cultured in the presence of IFNτ. Addition of OvIFNτ produced a rapid, dose-dependent decrease in reverse transcriptase (RT) activity (Example 11, Table 4). While concentrations as low as 0.62 ng/ml of IFNτ inhibited viral replication, much higher concentrations (40 ng/ml) having greater effects on RT-activity were without toxic effects on the cells. The results suggest that replication of the feline immunodeficiency virus was reduced significantly compared to control values when cells were cultured in the presence of OvIFNτ.

IFNτ appeared to exert no cytotoxic effect on the cells hosting the retrovirus. This was true even when IFNτ was present at 40 ng per ml of culture medium. This concentration of IFNτ is equivalent to about 8,000 antiviral units of alpha interferon, when IFNτ is assayed for its ability to protect Madin-Darby bovine kidney cells

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from lysis by vesicular stomatitis virus as described by
Pontzer, *et al.* (1988).

IFNτ was also tested for activity against HIV repli-
cation in human cells. Human peripheral lymphocytes,
5   which had been infected with HIV were treated with vary-
ing concentrations of IFNτ (Example 12). Replication of
HIV in peripheral blood lymphocytes was monitored by
reverse transcriptase activity in culture supernatants
over time. Over a range of concentrations of IFNτ pro-
10   duced significant anti-HIV effects (Example 12, Table 5).
A concentration of only 10 ng/ml resulted in over a 50%
reduction in RT activity after only six days. A concen-
tration of 500 ng/ml resulted in a 90% reduction in RT
activity within 10 days. Further, there was no evidence
15   of any cytotoxic effects attributable to the administra-
tion of IFNτ (Example 12, Table 5).

Further, the antiviral effects of IFNτ against HIV
were evaluated by treating human PBMC cells with various
amounts of either recombinant IFNτ or recombinant human
20   IFNα₂ at the time of infection with HIV (Example 18). The
data from these experiments (Example 18, Table 12) sup-
port the conclusion that, at similar concentrations, IFNα
and IFNτ are effective in reducing the replication of HIV
in human lymphocytes. However, treatment of cells with
25   IFNα₂ resulted in cytoxicity, whereas no such cytotoxity
was observed with treatment using IFNτ, even when IFNτ
was used at much higher concentrations. No cytotoxicity
was observed using IFNτ, even when IFNτ was used at 200
times the dosage of interferon-alpha II.
30   Both FIV and HIV reverse transcriptase themselves
were unaffected by IFNτ in the absence of PBL. There-
fore, the antiviral activity is not due to a direct
effect on the viral RT.

Interferon-τ has also been shown to inhibit Hepatitis B Virus DNA replication in hepatocytes (Example 18). A human cell derived from liver cells transfected with Hepatitis B Virus (HBV) was used to test the antiviral effects of IFNτ. The cells were treated with both the IFNα and IFNτ over a range of concentrations. Both IFNα and IFNτ reduced DNA production by approximately two-fold compared to the no interferon control.

To demonstrate that the effect of the interferons was specific to the infecting virus and not the result of an effect on general cell metabolism, the hepatocyte was examined for the effects of IFNα and IFNτ on hepatospecific mRNA production (Example 18). Two hepatocyte specific proteins, Apo E and Apo A1, were detected by hybridization analysis. There was no apparent reduction of mRNA production for either hepatospecific mRNA at concentrations up to 40,000 units/ml of either IFNα or IFNτ. Further, no evidence for hepatotoxicity with IFNτ was seen in this assay.

These results suggest that IFNτ is an effective antiviral agent against a wide variety of viruses, including both RNA and DNA viruses. One advantage of IFNτ over other interferons, such as IFNα, is that treatment with IFNτ does not appear to be associated with any cytotoxicity.

D. <u>Antiproliferative Properties of IFNτ.</u>

The effects of IFNτ on cellular growth have also been examined. In one analysis, anti-cellular growth activity was examined using a colony inhibition assay (Example 13). Human amnion (WISH) or MDBK cells were plated at low cell densities to form colonies originating from single cells. Dilutions of interferons were added to triplicate wells and the plates were incubated to allow colony formation. IFNτ inhibited both colony size

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and number in these assays.  IFNτ was more effective at
inhibiting cell proliferation of the human cell line
(WISH) than human IFNα.  The antiproliferative activity
of IFNτ was dose-dependent.  High concentrations of IFNτ

5    stopped proliferation, while cell viability was not
impaired.

     Based on cell cycle analysis, using flow cytometry,
IFNτ appears to inhibit progress of cells through S
phase.  These results demonstrate the antiproliferative

10   effect of IFNτ, and underscore its low cytotoxicity.

     The antiproliferative effects of IFNτ were also
studied for rat and bovine cell lines (Example 14).  The
rate of $^{3}$H-thymidine incorporation was used to assess the
rate of cellular proliferation.  The data obtained demon-

15   strate that IFNτ drastically reduced the rate of cellular
proliferation (Example 14, Table 7) for each tested cell
line.

     The antiproliferative activity and lack of toxicity
of IFNτ was further examined using a series of human

20   tumor cell lines (Example 15).  A variety of human tumor
cell lines were selected from the standard lines used in
NIH screening procedure for antineoplastic agents
(Pontzer, C.H., et al., (1991)).  At least one cell line
from each major neoplastic category was examined.

25

The following cell lines were obtained from American Type
Culture Collection (12301 Parklawn Dr., Rockville  MD
20852):

     NCI-H460   human lung large cell carcinoma;
30   DLD-1      human colon adenocarcinoma;
     SK-MEL-28  human malignant melanoma;
     ACHN       human renal adenocarcinoma;
     HL-60      human promyelocytic leukemia;
     H9         human T cell lymphoma;
35   HUT 78     human cutaneous T cell lymphoma; and
     MCF7       human breast adenocarcinoma.

As above, the antiproliferative activity was evaluated by measuring the rate of $^3$H-thymidine incorporation into cells which have been treated with IFNτ. Significant differences between treatments were assessed by an analysis of variance followed by Scheffe's F-test. Cell cycle analysis was performed by flow cytometry.

Examination of IFNτ inhibition of MCF7 (breast adenocarcinoma) proliferation demonstrated that IFNτ reduced MCF7 proliferation in a dose-dependent manner. A 50% reduction in $^3$H-thymidine was observed with 10,000 units/ml of IFNτ (Example 15, Table 8). This cell line had previously been found to be unresponsive to anti-estrogen treatment.

A comparison of the antiproliferative effects of IFNτ and IFNα was conducted using HL-60 (human promyelocytic leukemia) cells. Results with the promyelocytic leukemia HL-60 are typical of those obtained comparing IFNτ with human IFNα (Example 15). Concentrations as low as 100 units/ml of both IFNs produced significant (> 60%) growth reduction. Increasing amounts of IFNs further decreased tumor cell proliferation (Figure 4). At high doses of IFNα, but not IFNτ, was cytotoxic (Figure 5). Cell viability was reduced by approximately 80% by IFNα. By contrast, nearly 100% of the IFNτ-treated cells remained viable when IFNτ was applied at 10,000 units/ml. Thus, while both interferons inhibit proliferation, only IFNτ is without cytotoxicity. This lack of toxicity provides an advantage of IFNτ for use in *vivo* therapies.

The human cutaneous T cell lymphoma, HUT 78, responded similarly to HL-60 when treated with IFNτ (Example 15, Figure 9). Both OvIFNτ and rHuIFNα reduce HUT 78 cell growth, but IFNα demonstrated adverse effects on cell viability.

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The T cell lymphoma H9 was less sensitive to the
antiproliferative effects of IFNα than the tumor cell
lines described above. While IFNα was not toxic to the
H9 cells, it failed to inhibit cell division significant-
5      ly at any of the concentrations examined (Example 15,
Figure 10). In contrast, IFNτ was observed to reduce H9
growth by approximately 60%. Thus, only OvIFNτ is an
effective growth inhibitor of this T cell lymphoma.

In three additional tumor cell lines (NCI-H460, DLD-
10     1 and SK-MEL-28) IFNτ and IFNα were equally efficacious
antitumor agents. In the melanoma, SK-MEL-28, inhibition
of proliferation by IFNα was accomplished by a 13% drop
in viability, while IFNτ was not cytotoxic. In the
majority of tumors examined, IFNτ is equal or preferable
15     to IFNα as an antineoplastic agent against human tumors.

IFNτ exhibits antiproliferative activity against
human tumor cells without toxicity and is as potent or
more potent than human IFNα. Clinical trials of the
IFNα2s have shown them to be effective antitumor agents
20     (Dianzani, F., 1992; Krown, 1987). One therapeutic
advantage of IFNτ as a therapeutic is the elimination of
toxic effects seen with high doses IFNαs.

An additional application of the IFNτ is against
tumors like Kaposi's sarcoma (associated with HIV infec-
25     tion) where the antineoplastic effects of IFNτ are cou-
pled with IFNτ ability to inhibit retroviral growth.

The *in vivo* efficacy of interferon-τ treatment was
examined in a mouse system (Example 16). B16-F10 is a
syngeneic mouse transplantable tumor selected because of
30     its high incidence of pulmonary metastases (Poste, *et
al.*, 1981). Interferon treatment was initiated 3 days
after the introduction of the tumor cells. The *in vivo*
administration of IFNτ dramatically reduced B16-F10
pulmonary tumors. Thus, IFNτ appears to be an effica-
35     cious antineoplastic agent *in vivo* as well as *in vitro*.

## III. Interferon-τ Polypeptide Fragments, Protein Modeling and Protein Modifications.

The variety of IFNτ activities, its potency and lack of cytotoxicity, as taught by the present specification,

5 suggest the importance of structure/function anal is for this novel interferon. The structural basis for OvIFNτ function has been examined using six overlapping synthetic peptides corresponding to the entire OvIFNτ sequence (Figure 6). The corresponding polypeptides derived from

10 the ovine IFNτ sequence are presented as SEQ ID NO:15 to SEQ ID NO:20. Three peptides representing amino acids 1-37, 62-92 and 139-172 have been shown to inhibit IFNτ antiviral activity (Example 17). The peptides were effective competitors at concentrations of 300 $\mu$M and

15 above.

The C-terminal peptide of IFNτ, OvIFNτ (139-172), and the internal peptide OvIFNτ (62-92), inhibited IFNτ and rBoIFNα$_{II}$ antiviral activity to the same extent, while the N-terminal peptide OvIFNτ (1-37) was more effective

20 in inhibiting OvIFNτ antiviral activity. Dose-response data indicated that IFNτ (62-92) and IFNτ (139-172) inhibited IFNτ antiviral activity to similar extents. The same peptides that blocked IFNτ antiviral activity also blocked the antiviral activity of recombinant bovine

25 IFNα (rBoIFNα); recombinant bovine IFNγ was unaffected by the peptides. These two IFNτ peptides may represent common receptor binding regions for IFNτ and various IFNαs.

The two synthetic peptides OvIFNτ(1-37) and

30 OvIFNτ(139-172) also blocked OvIFNτ anti-FIV and anti-HIV activity (Example 17; Figures 11A and 11B). While both peptides blocked FIV RT activity, only the C-terminal peptide, OvIFNτ(139-172), appeared to be an efficient inhibitor of vesicular stomatitis virus activity on the

35 feline cell line, Fc9.

30

The above data taken together suggest that the C-terminal regions of type I interferons may bind to common site on the type I interferon receptor, while the N-terminal region may be involved in the elicitation of
5    unique functions.  These results suggest that portions of the IFNτ interferon molecule may be used to substitute regions of interferon alpha molecules.  For example, the region of an interferon alpha molecule that is responsible for increased cytotoxicity, relative to IFNτ treat-
10   ment, can be identified by substituting polypeptide regions derived from IFNτ for regions of an interferon alpha molecule.  Such substitutions can be carried out by manipulation of synthetic genes (see below) encoding the selected IFNτ and interferon alpha molecules, coupled to
15   the functional assays described herein (such as, antiviral, antiproliferative and cytoxicity assays).

Polyclonal anti-peptide antisera against the IFNτ peptides yielded similar results as the polypeptide inhibition studies, described above.  Antibodies directed
20   against the same three regions (OvIFNτ (1-37), IFNτ (62-92) and IFNτ (139-172)) blocked OvIFNτ function, confirming the importance of these three domains in antiviral activity (Example 17).  These peptides, although apparently binding to the interferon receptor, did not in and
25   of themselves elicit interferon-like effects in the cells.

The antiproliferative activity of IFNτ (Example 17, Table 11) involved a further region of the molecule, since IFNτ(119-150) was the most effective inhibitor of
30   OvIFNτ-induced reduction of cell proliferation.  This results suggests that the region of the molecule primarily responsible for inhibition of cell growth is the IFNτ(119-150) region.  This region of the IFNτ molecule may be useful alone or fused to other proteins (such as
35   serum albumin, an antibody or an interferon alpha poly-

peptide) as an antineoplastic agent. A conjugated protein between an N-terminal peptide derived from human interferon-α and serum albumin was shown to have anticellular proliferation activity (Ruegg, et al., 1990).

5    Finally, binding of $^{125}$I-OvIFNτ to its receptor on MDBK cells could be blocked by antisera to 4 of the 6 peptides; the 4 polypeptides representing amino acids 1-37, 62-92, 119-150 and 139-172 of OvIFNτ. This reflects the multiple binding domains as well as the functional

10   significance of these regions. Since different regions of IFNτ are involved in elicitation of different functions, modification of selected amino acids could potentially result in IFNτ-like interferons with selective biological activity.

15   The above data demonstrate the identification of synthetic peptides having four discontinuous sites on the OvIFNτ protein that are involved in receptor interaction and biological activity. In order to elucidate the structural relationship of these regions, modeling of the

20   three dimensional structure of IFNτ was undertaken. A three dimensional model would be useful in interpretation of existing data and the design of future structure/function studies.

Combining circular dichroism (CD) of both the full

25   length recombinant OvIFNτ to IFNβ (a protein of known three dimensional structure (Senda, et al., 1992)), a model of OvIFNτ has been constructed. The most striking feature of this model is that IFNτ falls into a class of proteins with a four-helix bundle motif. The CD spectra

30   of IFNτ was taken on an AVIV 60 S spectropolarimeter. Two different methods were employed for secondary structure estimations, the algorithm of Perczel, et al., (1991) and variable selection by W.C. Johnson, Jr. (1992).

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Secondary structure estimations of the spectra
indicate 70-75% alpha helix (characterized by minima at
222 and 208 nm and maximum at 190 nm). The variable
selection algorithm estimates the remainder of the mole-
5      cule to be 20% beta sheet and 10% turn. The Chang method
estimates the remainder to be 30% random coil. Alignment
of IFNτ and IFNβ sequences revealed homology between the
two molecules, specifically in the regions of known
helical structure in IFNβ. Sequence analysis of IFNτ
10     also showed that proposed helical regions possess an
apolar periodicity indicative of a four-helix bundle
motif.

The final modeling step was to apply the IFNβ x-ray
crystallographic coordinates of the IFNβ carbon backbone
15     to the IFNτ sequence. The functionally active domains of
IFNτ, identified above, were localized to one side of the
molecule and found to be in close spatial proximity.
This is consistent with multiple binding sites on IFNτ
interacting simultaneously with the type I IFN receptor.
20     The three dimensional modeling data coupled with the
function data described above, provides the ability to
introduce sequence variations into specific regions of
IFNτ to generate enhancement of selected functions (e.g.,
antiviral or anticellular proliferation) or the ability
25     to substitute a region(s) of selected function into other
interferon molecules (e.g., antiviral, antineoplastic, or
reduced cytotoxicity).

The construction of a synthetic gene for OvIFNτ is
described in Example 3. Briefly, a consensus amino
30     sequence was back-translated using optimal codon usage
for E. coli. The sequence was edited to include 20,
unique, restriction sites spaced throughout the length of
the construct. This 540 base pair synthetic gene se-
quence was divided into 11 oligonucleotide fragments.
35     Individual fragments were synthesized and cloned, either

single or double stranded, into either pTZ 19R, pTZ 18R
or pBluescript, amplified and fused. The synthetic
OvIFNτ construct was then cloned into a modified pIN-III-
ompA expression vector for expression in bacteria and

5    also cloned into a yeast expression plasmid. A similarly
constructed human IFNτ synthetic gene (SEQ ID NO:3) has
been designed, constructed and expressed in yeast cells.

Expression of the OvIFNτ synthetic gene in yeast
(Example 4) allowed over production of recombinant IFNτ

10   in *S. cerevisiae*: large quantities (5-20 mg/l) of recom-
binant IFNτ can be purified from soluble yeast extract
using sequential ion exchange and molecular sieve chroma-
tography. Recombinant IFNτ purified in this fashion
exhibited potent antiviral activity (2 to 3 X $10^8$

15   units/mg) similar to native OvIFNτ.

The synthetic gene construct facilitates introduc-
tion of mutations for possible enhancement of antitumor
(anticellular proliferative) and antiviral activities.
Further, the disparate regions of the molecule responsi-

20   ble for different functions allows for separate manipula-
tion of different functions. For example, two deletion
mutants, OvIFNτ(1-155) and OvIFNτ(1-166), have been
constructed to examine the role of carboxy terminal
sequences in IFNτ molecules.

25   Additional mutant IFNτ molecules have been con-
structed to identify residues critical for antiprolifera-
tive activity. For example, one particular residue, Tyr
123 has been implicated in the anticellular proliferative
activity of IFNα (McInnes, et al., 1989). The equivalent

30   of Tyr 123 in IFNτ is contained within peptide
OvIFNτ(119-150): this polypeptide inhibits OvIFNτ and
human IFNα antiproliferative activity. Mutations con-
verting Tyr 123 to conservative (Trp) and nonconservative
(Asp) substitutions have been generated, as well as

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mutant sequences having deletion of this residue. The codon for Tyr 123 is located within an SspI site; elimination of this site has been used for screening. The antiproliferative activity of these mutant IFNτ is evalu-
5    ated as described herein.

Synthetic peptides can be generated which correspond to the IFNτ polypeptides of the present invention. Synthetic peptides can be commercially synthesized or prepared using standard methods and apparatus in the art
10   (Applied Biosystems, Foster City CA).

Alternatively, oligonucleotide sequences encoding peptides can be either synthesized directly by standard methods of oligonucleotide synthesis, or, in the case of large coding sequences, synthesized by a series of clon-
15   ing steps involving a tandem array of multiple oligonucleotide fragments corresponding to the coding sequence (Crea; Yoshio et al.; Eaton et al.). Oligonucleotide coding sequences can be expressed by standard recombinant procedures (Maniatis et al.; Ausubel et al.).
20   The biological activities of the interferon-τ polypeptides described above can be exploited using either the interferon-τ polypeptides alone or conjugated with other proteins (see below).

25   IV.   Production of Fusion Proteins.
In another aspect, the present invention includes interferon-τ or interferon-τ-derived polypeptides covalently attached to a second polypeptide to form a fused, or hybrid, protein. The interferon-τ sequences making up
30   such fused proteins can be recombinantly produced interferon-τ or a bioactive portion thereof, as described above.

For example, where interferon-τ is used to inhibit viral expression, the polypeptides presented as SEQ ID
35   NO:10 and SEQ ID NO:20 may be advantageously fused with a

soluble peptide, such as, serum albumin, an antibody (e.g., specific against an virus-specific cell surface antigen), or an interferon alpha polypeptide. Other examples of fusion proteins include (i) replacing toxici-
5    ty-associated regions of interferon-α with the interfer-on-τ regions SEQ ID NO:5 and SEQ ID NO:15, and (ii) fusion proteins containing the interferon-τ regions SEQ ID NO:9 and SEQ ID NO:19 as anticellular proliferation agents.

10    The fused proteins of the present invention may be formed by chemical conjugation or by recombinant tech-niques. In the former method, the interferon-τ and second selected polypeptide are modified by conventional coupling agents for covalent attachment. In one exempla-
15    ry method for coupling soluble serum albumin to an inter-feron-τ polypeptide, serum albumin is derivatized with N-succinimidyl-S-acetyl thioacetate (Duncan), yielding thiolated serum albumin. The activated serum albumin polypeptide is then reacted with interferon-τ derivatized
20    with N-succinimidyl 3-(2-pyridyldithio) propionate (Cum-ber), to produce the fused protein joined through a disulfide linkage.

As an alternative method, recombinant interferon-τ may be prepared with a cysteine residue to allow disul-
25    fide coupling of the interferon-τ to an activated ligand, thus simplifying the coupling reaction. An interferon-τ expression vector, used for production of recombinant interferon-τ, can be modified for insertion of an inter-nal or a terminal cysteine codon according to standard
30    methods of site-directed mutagenesis (Ausubel, et al.).

In one method, a fused protein is prepared recombi-nantly using an expression vector in which the coding sequence of a second selected polypeptide is joined to the interferon-τ coding sequence. For example, human
35    serum albumin coding sequences can be fused in-frame to

36

the coding sequence of an interferon-τ polypeptide, such
as, SEQ ID NO:9. The fused protein is then expressed
using a suitable host cell. The fusion protein may be
purified by molecular-sieve and ion-exchange chromatogra-
5    phy methods, with additional purification by polyacryl-
amide gel electrophoretic separation and/or HPLC chroma-
tography, if necessary.

It will be appreciated from the above how interfer-
on-τ-containing fusion proteins may be prepared. One
10   variation on the above fusion is to exchange positions of
the interferon-τ and selected second protein molecules in
the fusion protein (e.g., carboxy terminal versus amino
terminal fusions). Further, internal portions of a
native interferon-τ polypeptide (for example, amino acid
15   regions of between 15 and 172 amino acids) can be assem-
bled into polypeptides where two or more such interferon-
τ portions are contiguous that are normally discontinuous
in the native protein.


20       V.  Antibodies Reactive with Interferon-τ.
Fusion proteins containing the polypeptide antigens
of the present invention fused with the glutathione-S-
transferase (Sj26) protein can be expressed using the
pGEX-GLI vector system in *E. coli* JM101 cells. The fused
25   Sj26 protein can be isolated readily by glutathione
substrate affinity chromatography (Smith). Expression
and partial purification of IFNτ proteins is described in
(Example 20), and is applicable to any of the other
soluble, induced polypeptides coded by sequences de-
30   scribed by the present invention.

Insoluble GST (sj26) fusion proteins can be  puri-
fied by preparative gel electrophoresis.

Alternatively, IFNτ-β-galactosidase fusion proteins
can be isolated as described in Example 19.

Also included in the invention is an expression
vector, such as the lambda gt11 or pGEX vectors described
above, containing IFNτ coding sequences and expression
control elements which allow expression of the coding

5    regions in a suitable host.  The control elements gener-
ally include a promoter, translation initiation codon,
and translation and transcription termination sequences,
and an insertion site for introducing the insert into the
vector.

10   The DNA encoding the desired polypeptide can be
cloned into any number of vectors (discussed above) to
generate expression of the polypeptide in the appropriate
host system.  These recombinant polypeptides can be
expressed as fusion proteins or as native proteins.  A

15   number of features can be engineered into the expression
vectors, such as leader sequences which promote the
secretion of the expressed sequences into culture medium.
Recombinantly produced IFNτ, and polypeptides derived
therefrom, are typically isolated from lysed cells or

20   culture media.  Purification can be carried out by meth-
ods known in the art including salt fractionation, ion
exchange chromatography, and affinity chromatography.
Immunoaffinity chromatography can be employed using
antibodies generated against selected IFNτ antigens.

25   In another aspect, the invention includes specific
antibodies directed against the polypeptides of the
present invention.  Typically, to prepare antibodies, a
host animal, such as a rabbit, is immunized with the
purified antigen or fused protein antigen.  Hybrid, or

30   fused, proteins may be generated using a variety of
coding sequences derived from other proteins, such as β-
galactosidase or glutathione-S-transferase.  The host
serum or plasma is collected following an appropriate
time interval, and this serum is tested for antibodies

35   specific against the antigen.  Example 20 describes the

38

production of rabbit serum antibodies which are specific
against the IFNτ antigens in a Sj26/IFNτ hybrid protein.
These techniques can be applied to the all of the IFNτ
molecules and polypeptides derived therefrom.

5         The gamma globulin fraction or the IgG antibodies of
immunized animals can be obtained, for example, by use of
saturated ammonium sulfate or DEAE Sephadex, or other
techniques known to those skilled in the art for produ-
cing polyclonal antibodies.

10        Alternatively, purified protein or fused
protein may be used for producing monoclonal antibodies.
Here the spleen or lymphocytes from a animal immunized
with the selected polypeptide antigen are removed and
immortalized or used to prepare hybridomas by methods

15   known to those skilled in the art (Harlow, et al.).  Lym-
phocytes can be isolated from a peripheral blood sample.
Epstein-Barr virus (EBV) can be used to immortalize human
lymphocytes or a fusion partner can be used to produce
hybridomas.

20        Antibodies secreted by the immortalized cells are
screened to determine the clones that secrete antibodies
of the desired specificity, for example, by using the
ELISA or Western blot method (Ausubel et al.).  Experi-
ments performed in support of the present invention have

25   yielded four hybridomas producing monoclonal antibodies
specific for ovine IFNτ have been isolated.

          Antigenic regions of polypeptides are generally
relatively small, typically 7 to 10 amino acids in
length.  Smaller fragments have been identified as anti-

30   genic regions.  Interferon-τ polypeptide antigens are
identified as described above.  The resulting DNA coding
regions can be expressed recombinantly either as fusion
proteins or isolated polypeptides.

          In addition, some amino acid sequences can be conve-

35   niently chemically synthesized (Applied Biosystems,

Foster City CA). Antigens obtained by any of these
methods may be directly used for the generation of anti-
bodies or they may be coupled to appropriate carrier
molecules. Many such carriers are known in the art and
5   are commercially available (e.g., Pierce, Rockford IL).

Antibodies reactive with IFNτ are useful, for exam-
ple, in the analysis of structure/function relationships.


VI.   Utility
10   A.   Reproductive.

Although IFNτ bears some similarity to the IFNα
family based on structure and its potent antiviral prop-
erties, the IFNαs do not possess the reproductive proper-
ties associated with IFNτ. Also, recombinant bovine IFNα
15   has little or no effect on interestrous interval compared
to IFNτ (Davis, et al., 1992).

Therefore, although IFNτ has some structural simi-
larities to other interferons, it has very distinctive
properties of its own: for example, the capability of
20   significantly influencing the biochemical events of the
estrous cycle.

The human IFNτ of the present invention can be used
in methods of enhancing fertility and prolonging the life
span of the corpus luteum in female mammals as generally
25   described in Hansen, et al., herein incorporated by
reference. Further, the human interferon-τ of the pres-
ent invention could be used to regulate growth and devel-
opment of uterine and/or fetal-placental tissues. The
human IFNτ is particularly useful for treatment of hu-
30   mans, since potential antigenic responses are less likely
using such a same-species protein.


B.   Antiviral Properties.

The antiviral activity of IFNτ has broad therapeutic
35   applications without the toxic effects that are usually

associated with IFNαs.  Although the presence of IFNτ in culture medium inhibited reverse transcriptase activity of the feline immunodeficiency virus (Example 11), this is not due to a direct effect of IFNτ on the FIV.  Rath-
5    er, IFNτ appears to induce the host cell to produce a factor(s) which is inhibitory to the reverse transcriptase of the virus.

IFNτ was found to exert its antiviral activity without adverse effects on the cells:  no evidence of
10   cytotoxic effects attributable to the administration of IFNτ was observed.  It is the lack of cytotoxicity of IFNτ which makes it extremely valuable as an *in vivo* therapeutic agent.  This lack of cytotoxicity sets IFNτ apart from most other known antiviral agents and all
15   other known interferons.

Formulations comprising the IFNτ compounds of the present invention can be used to inhibit viral replication.

The human IFNτ of the present invention can be
20   employed in methods for affecting the immune relationship between fetus and mother, for example, in preventing transmission of maternal viruses (e.g., HIV) to the developing fetus.  The human interferon-τ is particularly useful for treatment of humans, since potential antigenic
25   responses are less likely using a homologous protein.

C.   Anticellular Proliferation Properties.

IFNτ exhibits potent anticellular activity.  IFNτ can also be used to inhibit cellular growth without the
30   negative side effects associated with other interferons which are currently known.  Formulations comprising the IFNτ compounds of the subject invention can be used to inhibit, prevent, or slow tumor growth.

The development of certain tumors is mediated by
35   estrogen.  Experiments performed in support of the pres-

ent invention indicate that IFNτ can suppress estrogen receptor numbers. Therefore, IFNτ can be used in the treatment or prevention of estrogen-dependent tumors.

D.   Interfering with the Binding of Interferons to Receptors.

IFNτ appears to interact with the Type I IFN receptor via several epitopes on the molecule, and these regions either separately or in combination may affect distinct functions of IFNτ differently.

The polypeptides of the present invention are useful for the selective inhibition of binding of interferons to the interferon receptor. Specifically, as described herein, certain of the disclosed peptides selectively inhibit the antiviral activity of IFNτ while others inhibit the antiproliferative activity. Combinations of these peptides could be used to inhibit both activities. Advantageously, despite binding to the interferon receptor and blocking IFNτ activity, these peptides do not, themselves, elicit the antiviral or antiproliferative activity.

Therefore, such polypeptides can be used as immuno-regulatory molecules when it is desired to prevent immune responses triggered by interferon molecules. These peptides could be used as immunosuppressants to prevent, for example, interferon-mediated immune responses to tissue transplants. Other types of interferon mediated responses may also be blocked, such as the cytotoxic effects of alpha interferon.

E.   Pharmaceutical Compositions.

IFNτ proteins can be formulated according to known methods for preparing pharmaceutically useful composi-tions. Formulations comprising interferons or interfer-

on-like compounds have been previously described (for
example, Martin, 1976). In general, the compositions of
the subject invention will be formulated such that an
effective amount of the IFNτ is combined with a suitable
5    carrier in order to facilitate effective administration
of the composition.

The compositions used in these therapies may also be
in a variety of forms. These include, for example,
solid, semi-solid, and liquid dosage forms, such as
10    tablets, pills, powders, liquid solutions or suspensions,
liposomes, suppositories, injectable, and infusible
solutions. The preferred form depends on the intended
mode of administration and therapeutic application. The
compositions also preferably include conventional pharma-
15    ceutically acceptable carriers and adjuvants which are
known to those of skill in the art. Preferably, the
compositions of the invention are in the form of a unit
dose and will usually be administered to the patient one
or more times a day.

20    IFNτ, or related polypeptides, may be administered
to a patient in any pharmaceutically acceptable dosage
form, including intravenous, intramuscular, intrale-
sional, or subcutaneous injection. Specifically, compo-
sitions and methods used for other interferon compounds
25    can be used for the delivery of these compounds.

One primary advantage of the compounds of the sub-
ject invention, however, is the extremely low cytotoxici-
ty of the IFNτ proteins. Because of this low cytotoxici-
ty, it is possible to administer the IFNτ in concentra-
30    tions which are greater than those which can generally be
utilized for other interferon (e.g., IFNα) compounds.
Thus, IFNτ can be administered at rates from about $5 \times 10^4$
to $20 \times 10^6$ units/day to about $500 \times 10^6$ units/day or
more. In a preferred embodiment, the dosage is about $10^6$

units/day. High doses are preferred for systemic admin-
istration. It should, of course, be understood that the
compositions and methods of this invention may be used in
combination with other therapies.

5      Once improvement of a patient's condition has oc-
curred, a maintenance dose is administered if necessary.
Subsequently, the dosage or the frequency of administra-
tion, or both, may be reduced, as a function of the
symptoms, to a level at which the improved condition is
10     retained. When the symptoms have been alleviated to the
desired level, treatment should cease. Patients may,
however, require intermittent treatment on a long-term
basis upon any recurrence of disease symptoms.

The compositions of the subject invention can be
15     administered through standard procedures to treat a
variety of cancers and viral diseases including those for
which other interferons have previously shown activity.
See, for example, Finter, *et al*. (1991); Dianzani, *et al*.
(1992); Francis, *et al*. (1992) and U.S. Patent Nos.
20     4,885,166 and 4,975,276. However, as discussed above,
the compositions of the subject invention have unique
features and advantages, including their ability to treat
these conditions without toxicity.

25     F.   <u>Treatment of Skin Disorders</u>.

Disorders of the skin can be treated intralesionally
using IFNτ, wherein formulation and dose will depend on
the method of administration and on the size and severity
of the lesion to be treated. Preferred methods include
30     intradermal and subcutaneous injection. Multiple injec-
tions into large lesions may be possible, and several
lesions on the skin of a single patient may be treated at
one time. The schedule for administration can be deter-
mined by a person skilled in the art. Formulations

44

designed for sustained release can reduce the frequency
of administration.

G.    Systemic Treatment.

5        Systemic treatment is essentially equivalent for all
applications.  Multiple intravenous or subcutaneous doses
are possible, and in the case of implantable methods for
treatment, formulations designed for sustained release
are particularly useful.  Patients may also be treated

10   using implantable subcutaneous portals, reservoirs, or
pumps.

H.    Regional Treatment.

Regional treatment with the IFNτ polypeptides of the

15   present invention is useful for treatment of cancers in
specific organs.  Treatment can be accomplished by intra-
arterial infusion.  A catheter can be surgically or
angiographically implanted to direct treatment to the
affected organ.  A subcutaneous portal, connected to the

20   catheter, can be used for chronic treatment, or an im-
plantable, refillable pump may also be employed.

The following examples illustrate, but in no way are
intended to limit the present invention.

25

Materials and Methods

Restriction endonucleases, T4 DNA ligase, T4 polynu-
cleotide kinase, Taq DNA polymerase, and calf intestinal
phosphatase were purchased from New England Biolabs

30   (Beverly, MA) or Promega Biotech (Madison, WI):  these
reagents were used according to the manufacturer's in-
struction.  For sequencing reactions, a "SEQUENASE DNA
II" sequencing kit was used (United States Biochemical
Corporation, Cleveland OH).  Immunoblotting and other

35   reagents were from Sigma Chemical Co. (St. Louis, MO) or

Fisher Scientific (Needham, MA). Nitrocellulose filters are obtained from Schleicher and Schuell (Keene, NH).

5 Synthetic oligonucleotide linkers and primers are prepared using commercially available automated oligonucleotide synthesizers (e.g., an ABI model 380B-02 DNA synthesizer (Applied Biosystems, Foster City, CA)). Alternatively, custom designed synthetic oligonucleotides may be purchased, for example, from Synthetic Genetics (San Diego, CA). cDNA synthesis kit and random priming

10 labeling kits are obtained from Boehringer-Mannheim Biochemical (BMB, Indianapolis, IN).

Oligonucleotide sequences encoding polypeptides can be either synthesized directly by standard methods of oligonucleotide synthesis, or, in the case of large

15 coding sequences, synthesized by a series of cloning steps involving a tandem array of multiple oligonucleotide fragments corresponding to the coding sequence (Crea; Yoshio et al.; Eaton et al.). Oligonucleotide coding sequences can be expressed by standard recombinant

20 procedures (Maniatis et al.; Ausubel et al.).

Alternatively, peptides can be synthesized directly by standard *in vitro* techniques (Applied Biosystems, Foster City CA).

Common manipulations involved in polyclonal and

25 monoclonal antibody work, including antibody purification from sera, are performed by standard procedures (Harlow et al.). Pierce (Rockford, IL) is a source of many antibody reagents.

Recombinant human IFNα (rHuIFNα) and rBoIFNγ was

30 obtained from Genentech Inc. (South San Francisco, CA). The reference preparation of recombinant human IFNα (rHuIFNα) was obtained from the National Institutes of Health: rHuIFNα is commercially available from Lee Biomolecular (San Diego, CA).

46

All tissue culture media, sera and IFNs used in this study were negative for endotoxin, as determined by assay with Limulus amebocyte lysate (Associates of Cape Cod, Woods Hole, MA) at a sensitivity level of 0.07 ng/ml.

5

General ELISA Protocol for Detection of Antibodies.

Polystyrene 96 well plates Immulon II (PGC) were coated with 5 $\mu$g/mL (100 $\mu$L per well) antigen in 0.1 M carb/bicarbonate buffer, pH 9.5. Plates were sealed with parafilm and stored at 4°C overnight.

10 Plates were aspirated and blocked with 300 uL 10% NGS and incubated at 37°C for 1 hr.

Plates were washed 5 times with PBS 0.5% "TWEEN-20".

Antisera were diluted in 0.1 M PBS, pH 7.2. The

15 desired dilution(s) of antisera (0.1 mL) were added to each well and the plate incubated 1 hours at 37°C. The plates was then washed 5 times with PBS 0.5% "TWEEN-20".

Horseradish peroxidase (HRP) conjugated goat anti-human antiserum (Cappel) was diluted 1/5,000 in PBS. 0.1

20 mL of this solution was added to each well. The plate was incubated 30 min at 37°C, then washed 5 times with PBS.

Sigma ABTS (substrate) was prepared just prior to addition to the plate.

25 The reagent consists of 50 mL 0.05 M citric acid, pH 4.2, 0.078 mL 30% hydrogen peroxide solution and 15 mg ABTS. 0.1 mL of the substrate was added to each well, then incubated for 30 min at room temperature. The reaction was stopped with the addition of 0.050 mL 5% SDS

30 (w/v). The relative absorbance is determined at 410 nm.

EXAMPLE 1

Reproductive Functions of IFN$\tau$

The effect of interferon-$\tau$ on the lifespan of the

35 corpus lutem was examined.

IFNτ was infused into uterine lumen of ewes at the concentrations given in Table 1. Recombinant human IFNα (rHuIFNα) was infused at similar concentrations. In addition, control animals, which received control pro-
5   teins, were also used. The life span of the corpus luteum was assessed by examination of interestrous inter- vals, maintenance of progesterone secretion, and inhibi- tion of prostaglandin secretion (Davis, *et al.*, *1992*).

10                            Table 1

Effect of Interferons on Reproductive Physiology

| Interferon | Treatment | Interestrous Interval (days) (Means) |
|---|---|---|
| Control | - | 17.3 |
| rHuIFNα | 100 μg/day | 16.0 |
| | 200 μg/day | 16.0 |
| | 2000 μg/day | 19.0 |
| OvIFNτ | 100 μg/day | 27.2 |

20

Comparison of the interestrous intervals for the control animals and for animals receiving OvIFNτ demon- strate a considerable lengthening of the interval, when IFNτ is administered at 100μg/day. On the other hand,
25   comparison of the interestrous interval for the control animal and for animals receiving recombinant human IFNα, demonstrated that rHuIFNα had no meaningful effect.

These results demonstrate that interferon-τ has the capability of significantly influencing the biochemical
30   events of the reproductive cycle.

EXAMPLE 2

Antiviral Properties of Interferon-τ at Various Stages of the Reproductive Cycle
35   Conceptus cultures were established using conceptus obtained from sheep at days 12 through 16 of the estrous

48

cycle. Antiviral activity of supernatant from each
conceptus culture was assessed using a cytopathic effect
assay (Familetti, *et al.*, 1981). Briefly, dilutions of
IFNτ or other IFNs were incubated with Madin-Darby bovine
5   kidney (MDBK) cells for 16-18 hours at 37°C. Following
incubation, inhibition of viral replication was deter-
mined in a cytopathic effect assay using vesicular stoma-
titis virus (VSV) as the challenge virus.

One antiviral unit caused a 50% reduction in de-
10   struction of the monolayer, relative to untreated MDBK
cells infected with VSV (control plates). Specific
activities were further evaluated using normal ovine
fibroblasts (Shnf) in a plaque inhibition assay (Lang-
ford, *et al.*, 1981). A minimum of three samples were
15   examined at each time point, and each sample was assayed
in triplicate. The results presented in Table 2 are
expressed as mean units/ml.

20                                    Table 2

### IFNτ Antiviral Activity of Conceptus Cultures and Allantoic and Amniotic Fluids

|  | Day | Samples | Units/ml |
|---|---|---|---|
| Conceptus Cultures | 10 | 9 | <3 |
| | 10 | 5 | 34 |
| | 13 | 4 | $4.5 \times 10^3$ |
| | 14 | 3 | $7.7 \times 10^3$ |
| | 16 | 12 | $2.0 \times 10^6$ |
| Allantoic Fluid | 60 | 4 | $1.4 \times 10^3$ |
| | 100 | 4 | 11 |
| | 140 | 3 | <3 |
| Amniotic Fluid | 60 | 3 | 22 |
| | 100 | 4 | <3 |

35

Culture supernatants had increasing antiviral activity associated with advancing development of the conceptus (Table 2).

5                          EXAMPLE 3

                   Expression of IFNτ in Bacteria

The amino acid coding sequence for OvIFNτ (Imakawa, et al., 1987) was used to generate a corresponding DNA coding sequence with codon usage optimized for expression
10  in E. coli.  Linker sequences were added to the 5' and 3' ends to facilitate cloning in bacterial expression vectors.  The nucleotide sequence was designed to include 19 unique restriction enzyme sites spaced evenly throughout the coding sequence (Figure 1).
15      The nucleotide sequence was divided into eleven oligonucleotide fragments ranging in sizes of 33 to 75 bases.  Each of the eleven oligonucleotides were synthesized on a 380-B 2-column DNA synthesizer (Applied Biosytems) and cloned single- or double-stranded into one of
20  the following vectors:  "pBLUESCRIPT$^+$(KS)" (Stratagene, LaJolla, CA), pTZ18R (Pharmacia, Piscataway, NJ), or pTZ19R (Pharmacia, Piscataway, NJ) cloning vectors.

The vectors were transformed into E. coli K. strain "XL1-BLUE" (recA1 endA1 gyrA96 thi hsdR17 ($r_k^-$, $m_k^+$) supE44
25  relA1 λ - (lac), {F', proAB, lac$^q$ZΔM15, Tn10(tet$^R$}) which is commercially available from Stratagene (LaJolla, CA). Transformed cells were grown in L broth supplemented with ampicillin (50 μg/ml).  Oligonucleotide cloning and fusion was performed using standard recombinant DNA
30  techniques.

Cloning vectors were cut with the appropriate restriction enzymes to insert the synthetic oligonucleotides.  The vectors were treated with calf intestine alkaline phosphatase (CIP) to remove terminal phosphate

50

groups. Oligonucleotides were phosphorylated and cloned, as either single- or double-stranded molecules, into the appropriate vector using T4 DNA ligase. When single-strands were introduced into cloning vectors, the second

5    strand was completed by the bacterial host following transfection.

For double-stranded cloning, oligonucleotides were first annealed with their synthetic complementary strand then ligated into the cloning vector. *E. coli* K12

10   strains SB221 or NM522 were then transformed with the ligation. *E. coli* strain GM119 was used for cloning when the methylation-sensitive *StuI* and *ClaI* restriction sites were involved. Restriction analyses were performed on isolated DNA at each stage of the cloning procedure.

15   Cloned oligonucleotides were fused into a single polynucleotide using the restriction digestions and ligations outlined in Figure 2. Oligonucleotide-containing-DNA fragments were typically isolated after electrophoretic size fractionation on low-melting point agarose

20   gels (Maniatis, *et al.*; Sambrook, *et al.*; Ausubel, *et al.*). The resulting IFNτ polynucleotide coding sequence spans position 16 through 531: a coding sequence of 172 amino acids.

The nucleotide sequence of the final polynucleotide

25   was confirmed by DNA sequencing using the dideoxy chain termination method.

The full length *StuI/SstI* fragment (540 bp; Figure 2) was cloned into a modified pIN III omp-A expression vector and transformed into a competent SB221 strain of

30   *E. coli*. For expression of the IFNτ protein, cells carrying the expression vector were grown in L-broth containing ampicillin to an OD (550 nm) of 0.1-1, induced with IPTG for 3 hours and harvested by centrifugation. Soluble recombinant IFNτ was liberated from the cells by

35   sonication or osmotic fractionation.

EXAMPLE 4

Expression of IFNτ in Yeast

The synthetic IFNτ gene, synthesized in Example 3, was flanked at the 5' end by an *StuI* restriction site and

5    at the 3' end by a *SacI* restriction site.


A.   Isolation of the Synthetic IFNτ Gene.

Two oligonucleotide primers (SEQ ID NO:13 and SEQ ID NO:14) were used to attach linkers to the synthetic IFNτ

10   gene using polymerase chain reaction.  The linker at the 5' end allowed the placement of the synthetic IFNτ gene in correct reading with the ubiquitin coding sequence present in the yeast cloning vector pBS24Ub (Chiron Corp., Emeryville, CA).  The linker also constructed a

15   ubiquitin-IFNτ junction region that allowed *in vivo* cleavage of the ubiquitin sequences from the IFNτ sequences.  The 5' oligonucleotide also encoded a *SacII* restriction endonuclease cleavage site.  The 3' oligonucleotide contained a *StuI* cleavage site.

20        The vector carrying the synthetic IFNτ gene (Example 3) was isolated from *E. coli* strain "XLI-BLUE" by the alkaline lysis method.  Isolated vector was diluted 500-fold in 10 mM Tris, pH 8.0/1 mM EDTA/10 mM NaCl.  The PCR reaction was performed in a 100 $\mu$l volume using *Taq* DNA

25   polymerase and primers SEQ ID NO:13/SEQ ID NO:14.  The amplified fragments were digested with *StuI* and *SacII*. These digested fragments were ligated into the *SacII* and *SmaI* sites of "pBLUESCRIPT+(KS)."

The resulting plasmid was named pBSY-IFNτ.  The DNA

30   sequence was verified using double stranded DNA as the template.


B.   Construction of the Expression Plasmid.

Plasmid pBSY-IFNτ was digested with *SacII* and *EcoRV*

35   and the fragment containing the synthetic IFNτ gene was

isolated. The yeast expression vector pBS24Ub (Sabin, et al.; Ecker, et al.) was digested with SalI. Blunt ends were generated using T4 DNA polymerase. The vector DNA was extracted with phenol and ethanol precipitated (Sam-

5 brook, et al., 1989). The recovered linearized plasmid was digested with SacII, purified by agarose gel electro-phoresis, and ligated to the SacII-EcoRV fragment isolat-ed from pBSY-IFNτ. The resulting recombinant plasmid was designated pBS24Ub-IFNτ.

10 The recombinant plasmid pBS24Ub-IFNτ was transformed into E. coli. Recombinant clones containing the IFNτ insert were isolated and identified by restriction enzyme analysis. Plasmid DNA from clones containing IFNτ coding sequences was used for transformation of S. cerevisiae

15 (Rothstein, 1986). Transformation mixtures were plated on uracil omission medium and incubated for 3-5 days at 30°C. Colonies were then streaked and maintained on uracil and leucine omission medium (Rothstein, 1986).

20 C.   Expression Experiments.

For small-scale expression, a single colony of S. cerevisiae AB116 containing pBS24Ub-IFNτ was picked from a leucine and uracil omission plate and grown at 30°C in YEP medium (1% yeast extract, 2% peptone) containing 1%

25 glucose for inducing conditions or 8% glucose for non-inducing conditions. Cell lysates were recovered and subjected to SDS-PAGE in 15% acrylamide, 0.4% bisacryla-mide (Sambrook, et al., 1989). The fractionated proteins were visualized by Coomassie blue staining.

30 Recombinant IFNτ was visualized specifically by immunoblotting with monoclonal antibody or polyclonal antiserum against ovine IFNτ upon electrotransfer of the fractionated cell extract to "NYTRAN" paper (Rothstein, 1986).

For large-scale expression, pBS24-IFNτ was grown for 24 hours at 30°C in 5 × uracil and leucine omission medium containing 8% glucose. This culture was then diluted 20-fold in YEP medium containing 1% glucose and

5 further incubated for another 24-36 hours.

Cells were harvested by centrifugation, washed in 50 mM Tris, pH 7.6,/1 mM EDTA and resuspended in wash buffer containing 1 mM PMSF. The cells were lysed using a Bead-beater apparatus (Biospec Products, Bartlesville, OK).

10 The lysate was spun at 43,000 × g for 20 minutes. The supernatant fraction was recovered and subjected to the purification protocol described below.

D.    Purification of r-IFNτ from Yeast Cell Lysate.

15 The supernatant was loaded on a 1 × 10 cm DEAE column and washed with 10 mM Tris, pH 8.0. Retained proteins were eluted with a 300 ml, 0 to 0.5 M NaCl gradient in 10 mM Tris, pH 8.0. Three-milliliter fractions were collected. Ten-microliter samples of frac-

20 tions 17-26 containing the recombinant (r-IFNτ) were electrophorectically separated on 15% SDS-polyacrylamide gels. The gels were stained with Coomassie blue.

Fractions 18, 19, and 20 contained largest amount of r-IFNτ. These fractions were loaded individually on a

25 1.5 × 90 cm Sephadex S-200 column and proteins were resolved in two peaks. Aliquots of each protein peak (25 μl) were electrophoretically separated on 15% SDS-poly-acrylamide gels and the proteins visualized with Coomas-sie staining.

30 Purified r-IFNτ-containing fractions were combined and the amount of r-IFNτ quantified by radioimmunoassay (Vallet, *et al.*, 1988). Total protein concentration was determined by using the Lowry protein assay (Lowry, *et al.*, 1951).

54

Microsequencing of purified r-IFNτ demonstrated
identity with native IFNτ through the first 15 amino
acids, confirming that the ubiquitin/r-IFNτ fusion pro-
tein was correctly processed *in vivo*.

5    Purified r-IFNτ exhibited 2 to 3 × $10^8$ units of
antiviral activity per milligram of protein ($n$ = 3 repli-
cate plates) which is similar to the antiviral activity
of IFNτ purified from conceptus-conditioned culture
medium (2 × $10^8$ U/mg).

10

EXAMPLE 5
Southern Blot Analysis of Human
High Molecular Weight DNA

Human venous blood samples from healthy donors were
15   collected in heparinized tubes and peripheral blood
lymphocytes were isolated by density-gradient centrifuga-
tion using a Ficoll-Isopaque gradient (1.077 g/ml) (Sigma
Chemical Co.). High molecular weight (HMW) DNA was
isolated from these cells (Sambrook, *et al.*, 1989).

20   Two 10 μg samples of HMW DNA were digested with the
restriction endonucleases *Hind*III or *Pst*I (Promega) for 2
hours at 37°C, and the DNA fragments electrophoretically
separated in a 0.8% agarose gel (Bio-Rad, Richmond, CA)
at 75 volts for 8 hours. The DNA fragments were trans-
25   ferred onto a nylon membrane (IBI-International Biotech-
nologies, Inc., New Haven, CT). The membrane was baked
at 80°C for 2 hours and incubated at 42°C for 4 hours in
the following prehybridization solution:  5 × SSC (1 ×
SSC is 0.15 M NaCl and 0.15 M sodium citrate), 50% vol/-
30   vol formamide, 0.6% (wt/vol) SDS, 0.5% (wt/vol) nonfat
dry milk, 20 mM Tris-HCl (pH 7.5), 4 mM EDTA, and 0.5
mg/ml single stranded herring sperm DNA (Promega).

The filter was then incubated in a hybridization
solution (5 × SSC, 20% vol/vol formamide, 0.6% (wt/vol)
35   SDS, 0.5% (wt/vol) nonfat dry milk, 20 mM Tris-HCl (pH

7.5), 4 mM EDTA, and 2 × 10⁸ cpm/ml ³²P-labelled OvIFNτ
cDNA (Imakawa, *et al.*, 1987)) for 18 hours at 42°C. The
filter was washed at 42°C for 15 minutes with 2 × SSC and
0.1% (wt/vol) SDS and exposed to X-ray film (XAR, Eastman

5    Kodak, Rochester, NY) at -80°C for 48 hours in the pres-
ence of an intensifying screen.

Autoradiography detected a hybridization signal at
approximately 3.4 kb in DNA digested with *Pst*I and a
slightly smaller (≈ 3.0 kb) fragment in the *Hind*III

10   digested DNA. These results indicate the presence of
human DNA sequences complementary to the OvIFNτ cDNA
probe.


### EXAMPLE 6

15   <u>Isolation of Partial Sequence of</u>
<u>Human IFN cDNA by PCR</u>

Two synthetic oligonucleotides (each 25-mer), corre-
sponding to the sequence 231 to 255 (contained in SEQ ID
NO:13) and 566 to 590 (contained in SEQ ID NO:14) of

20   OvIFNτ cDNA (numbering relative to the cap site, Imakawa,
*et al.*, 1987) were synthesized. These primers contained,
respectively, cleavage sites for the restriction endonu-
cleases *Pst*I and *Eco*RI. SEQ ID NO:13 was modified to
contain the *Eco*RI site, which begins at position 569.

25   DNA was isolated from approximately 1 × 10⁵ plaque
forming units (pfu) of the following two cDNA libraries:
human term placenta (Clontech, Inc., Palo Alto, CA) and
human term cytotrophoblast (Dr. J.F. Strauss, University
of Pennsylvania, Philadelphia PA). The DNA was employed

30   in polymerase chain reaction (PCR) amplifications (Mul-
lis; Mullis, *et al.*; Pekin Elmer Cetus Corp. Norwalk CT).
Amplification reactions were carried out for 30 cycles
(45°C, 1m; 72°C, 2m; 94°C, 1m) (thermal cycler and re-
agents, Perkin Elmer Cetus) using primers SEQ ID

35   NO:13/SEQ ID NO:14.

56

Amplification products were electrophoretically separated (100 volts in a 1.5% agarose gel (Bio-Rad)) and transferred onto a nylon membrane (IBI). The membrane was baked at 80°C for 2 hours and prehybridized and

5    hybridized with $^{32}$P-labelled OvIFNτ cDNA as described above. The membrane was washed in 5 × SSC/0.1% (wt/vol) SDS for 5 minutes at 42°C and in 2 × SSC/0.1% (wt/vol) SDS for 2 minutes at 42°C. It was then exposed at -80°C to "XAR" (Eastman Kodak) X-ray film for 24 hours in the

10   presence of an intensifying screen. An amplification product that hybridized with the labelled probe DNA was detected.

PCR was performed again as directed above. Amplified products were digested with the restriction endonu-

15   cleases EcoRI and PstI (Promega) for 90 minutes at 37°C. The resulting DNA fragments were electrophoretically separated as described above and the band containing the IFNτ amplification product was excised from the gel. DNA fragments were recovered by electroelution, subcloned

20   into EcoRI/PstI digested-dephosphorylated plasmid pUC19 and transformed into E. coli strain JM101 (Promega) by calcium chloride method (Sambrook, et al., 1989). The plasmids were isolated and the inserted amplification product sequenced using the dideoxy termination method

25   (Sanger, et al., 1977; "SEQUENASE" reactions, United States Biochemical, Cleveland, OH). Nucleotide sequences were determined, and comparison of these as well as the deduced amino acid sequences to other IFN sequences were performed using DNA Star Software (Madison, WI).

30   Comparison of the sequences of these clones revealed three different clones: from the human placental li-brary, Clone 15 (306 bp) and Clone 21 (315 bp), which exhibit 95% identity in their nucleotide sequences; from the cytotrophoblast library clone CTB 35 (301 basepairs),

which shares 95% and 98% identity with Clone 15 and Clone 21, respectively.

5                              EXAMPLE 7

Isolation of Full-Length Human IFNτ Gene

Ten micrograms PBMC HMW DNA was digested with re-
striction endonuclease EcoRI and subjected to electropho-
retic analysis in a 0.8% agarose gel.  A series of sam-
10   ples containing ranges of DNA fragments sized 1.5 to 10
kb (e.g., 1.5 to 2.5 kb, 2.5 kb to 3 kb) were excised
from the gel.  The DNAs were electroeluted and purified.
Each DNA sample was amplified as described above using
the OvIFNτ primers.  The DNA molecules of any sample that
15   yielded a positive PCR signal were cloned into λgt11 (the
subgenomic λgt11 library).

A.      PCR Identification of Clones Containing Se-
        quences Complementary to OvIFNτ.

20       The λgt11 phage were then plated for plaques and
plaque-lift hybridization performed using the $^{32}$P-labelled
OvIFNτ cDNA probe.  Approximately 20 clones were identi-
fied that hybridized to the probe.

Plaques that hybridized to the probe were further
25   analyzed by PCR using the OvIFNτ primers described above.
Six plaques which generated positive PCR signals were
purified.  The phage DNA from these clones was isolated
and digested with EcoRI restriction endonuclease.  The
DNA inserts were subcloned into pUC19 vectors and their
30   nucleotide sequences determined by dideoxy nucleotide
sequencings.

58

B.    Hybridization Identification of Clones Contain-
      ing Sequences Complementary to PCR-Positive
      Phage.

          Recombinant phage from the λgt11 subgenomic library

5   were propagated in *E. coli* Y1080 and plated with *E. coli*
    Y1090 at a density of about 20,000 plaques/150 mm plate.
    The plates were overlaid with duplicate nitrocellulose
    filters, which were hybridized with a $^{32}$P-labelled probe
    from one of the six human IFNτ cDNA clones isolated

10  above.   Three clones giving positive hybridization sig-
    nals were further screened and purified.   The phage DNAs
    were isolated, digested with *EcoRI*, subcloned into pUC19
    vector and sequenced.   The three clones yielded sequence
    information for over 800 bases relative to cap site

15  (clones were sequenced in both orientations).   The nucle-
    ic acid sequence information is presented as SEQ ID NO:11
    and the predicted protein coding sequence is presented as
    SEQ ID NO:12.   Comparison of the predicted mature protein
    sequence (SEQ ID NO:12) of this gene to the predicted

20  protein sequence of OvIFNτ is shown in Figure 3.


                         EXAMPLE 8

                   Analysis of the Presence of
                     HuIFNτ mRNA by RT-PCR

25        Human placental cDNA libraries and an ovine cDNA
    library, constructed from day 15-16 conceptuses, were
    analyzed by hybridization to the OvIFNτ cDNA probe,
    described above.   cDNAs were size-fractionated on agarose
    gels and transferred to filters (Maniatis, *et al.*; Sam-

30  brook, *et al.*).   Southern blot analysis with OvIFNτ probe
    showed that the autoradiographic signals from human cDNA
    libraries were approximately 1/100 of the signal obtained
    using the OvIFNτ cDNA library.

          The presence of HuIFNτ mRNA in human term placenta

35  and amniocytes (26 weeks, 2 million cells) was analyzed

by using reverse transcriptase-PCR (RT-PCR) method (Clontech Laboratories, Palo Alto CA).

Total cellular RNA (tcRNA) isolated from human placenta, amniocytes and ovine conceptuses were reverse transcribed using the primer SEQ ID NO:14. The primer SEQ ID NO:13 was then added to the reaction and polymerase chain reaction carried out for 40 cycles. The PCR products were size fractionated on agarose gels and transferred to filters. The DNA on the filters was hybridized with $^{32}$P-labelled OvIFN$\tau$ and HuIFN$\tau$ cDNAs. The results of these analyses demonstrate the presence of human IFN$\tau$ mRNA in the feto-placental annex. The aminocytes also expressed the messages corresponding to OvIFN$\tau$ primers and human probe.

In addition, a RT-PCR analysis for the presence of HuIFN$\tau$ was applied to the tcRNA isolated from human adult lymphocytes. A densitometric analysis revealed that IFN$\tau$ mRNA exists in lymphocytes.


## EXAMPLE 9
### *In Situ* Hybridization

A.    Tissue

Slides of semiserial 5-$\mu$ paraffin embedded sections from four healthy, different term and first trimester human placentas were examined.


B.    cRNA Probe Preparation

From the cDNA clone isolated from OvIFN$\tau$ amplified library a fragment corresponding to the OvIFN$\tau$ cDNA bases #77-736 (base #1 is cap site; open reading frame of OvIFN$\tau$ cDNA is base #81-665; Figure 7) was subcloned into the transcription vector, pBS (New England Biolabs). Several pBS clones were isolated, subcloned, and their nucleotides sequenced. From this clone a 3′ fragment

60

(bases #425-736) was excised using the restriction endon-
ucleases *Nla*IV and *Eco*RI and subcloned into the tran-
scription vector pBS.  This vector was designated
pBS/OvIFNτ.

5        After linearization of the pBS/OvIFNτ plasmid, an
antisense cRNA probe was synthesized by *in vitro* tran-
scription (Sambrook, *et al.*, 1989) using $T_7$ RNA polymerase
(Stratagene).  A trace amount of $^3$H-CTP (NEN-DuPont,
Cambridge, MA) was used in the transcription reaction.

10      dUTP labeled with digoxigenin (Boehringer-Mannheim,
Indianapolis, IN) was incorporated into the cRNA and
yield was estimated through TCA precipitation and scin-
tillation counting.


15       C.   Hybridization
         *In situ* hybridization was performed using the anti-
sense RNA probe, as described by Lawrence, *et al.* (1985)
with the following modifications.  Deparaffinized and
hydrated sections were prehybridized for 10 minutes at
20      room temperature in phosphate buffered saline (PBS)
containing 5 mM $MgCl_2$.  Nucleic acids in the sections were
denatured for 10 minutes at 65°C in 50% formamide/2 ×
SSC.  Sections were incubated overnight at 37°C with a
hybridization cocktail (30 μl/slide) containing 0.3 μg/ml
25      digoxigenin-labelled cRNA probe and then washed for 30
minutes each at 37°C in 50 formamide/1 × SSC.  Final
washes were performed for 30 minutes each at room temper-
ature in 1 × SSC and 0.1 × SSC.  The sections were
blocked for 30 minutes with 0.5% Triton X-100 (Sigma) and
30      0.5% non-fat dry milk.
         Hybridization signal was detected using purified
sheep antidioxigenin Fab fragments conjugated to alkaline
phosphatase (Boehringer-Mannheim).  After unbound anti-
body was removed, nitroblue tetrazolium/5-bromo-4-chloro-

3-indolyl-phosphate substrate (Promega) and levamisole
(Bector Laboratories, Burlingame, CA) were added for
signal detection via colorimetric substrate generation.
The tissues were counterstained in methyl green (Sigma),
5    dehydrated, and mounted.

As a control, some tissue sections were pretreated
with 100 $\mu$g/ml of pancreatic RNaseA (Sigma) for 30 min-
utes at 37°C.  The RNase was inactivated on the slide
with 400 units of RNase inhibitor (Promega).  The slides
10   were then washed twice in 250 ml of PBS/5 mM MgCl$_2$.  In
other control experiments, tRNA (Sigma) was substituted
for the digoxigenin probes.

Specific hybridization was observed in all term and
first trimester placental tissues in three separate
15   experiments with various OvIFN$\tau$ cRNA probe concentrations
and blocking reagents.

First trimester placental villi composed of an outer
layer of syncytiotrophoblast, an underlying layer of
cytotrophoblast, and a central stromal region with vari-
20   ous types of mesenchymal cells, displayed the highest
transcript level of IFN$\tau$ in the cytotrophoblast cells.
Less intense but detectable levels were present in both
the syncytiotrophoblast and stromal cells.  A similar
pattern of transcript expression was demonstrated in the
25   placental villi of term tissue but the level of signal
detection was low.  First trimester extravillous tropho-
blast displayed the highest amount of message and stained
positive when present in the maternal blood spaces.

30                          EXAMPLE 10
                    Antiviral Activity of IFN$\tau$
The relative specific activity of OvIFN$\tau$, purified
to homogeneity, was evaluated in antiviral assays.  The
antiviral assays were performed essentially as described

62

above in Example 2.    Specific activities are expressed in
antiviral units/mg protein obtained from antiviral assays
using either Madin-Darby bovine kidney (MDBK) cells or
sheep normal fibroblasts (Shnf).    All samples were as-
5    sayed simultaneously to eliminate interassay variability.
The results, presented in Table 3, are the means of four
determinations where the standard deviation was less than
10% of the mean.

10

### Table 3

### Antiviral Activity of IFNτ and Known IFNs

15

| | Specific Activities | |
|---|---|---|
| | MDBK | Shnf |
| OvIFNτ | $2 \times 10^8$ | $3 \times 10^8$ |
| rBoIFNα | $6 \times 10^7$ | $1 \times 10^7$ |
| rBoIFNγ | $4.5 \times 10^6$ | $3 \times 10^6$ |
| NIH rHuIFNα | $2.2 \times 10^8$ | $2.2 \times 10^8$ |
| rHuIFNα | $2.9 \times 10^5$ | $4.3 \times 10^5$ |

20

IFNτ had a higher specific activity than either
rBoIFNα or rBoIFNγ (Table 3).    The NIH standard prepara-
25    tion of rHuIFNα had a similar specific activity, while a
commercial preparation of rHuIFNα exhibited low specific
antiviral activity.    Comparable relative antiviral activ-
ity was demonstrated using either bovine or ovine cells.

30                          EXAMPLE 11
### Anti-Retroviral Activity and Cytotoxic
### Effects of IFNτ
Highly purified OvIFNτ was tested for anti-retrovi-
ral and cytotoxic effects on feline peripheral blood
35    lymphocytes exposed to the feline immunodeficiency retro-
virus.    This lentivirus produces a chronic AIDS-like

syndrome in cats and is a model for human AIDS (Pederson, *et al.*, 1987). Replication of the virus in peripheral blood lymphocytes is monitored by reverse transcriptase activity in culture supernatants over time. The data
5  from these assays are presented in Table 4.

Table 4

Effect of OvIFNτ on FIV Replication

| IFNτ Concentration (ng/ml) | RT Activity (cpm/ml) | | | | |
|---|---|---|---|---|---|
| **Experiment 1** | **Harvest Days** | | | | |
| | Day 2 | Day 5 | Day 8 | Day 12 | Day 15 |
| 0.00 | 93,908 | 363,042 | 289,874 | 171,185 | 125,400 |
| 0.62 | 77,243 | 179,842 | 172,100 | 218,281 | 73,039 |
| 1.25 | 94,587 | 101,873 | 122,216 | 71,916 | 50,038 |
| 2.50 | 63,676 | 72,320 | 140,783 | 75,001 | 36,105 |
| 5.00 | 69,348 | 82,928 | 90,737 | 49,546 | 36,299 |
| **Experiment 2** | **Harvest Days** | | | | |
| | Day 2 | Day 5 | Day 8 | Day 13 | Day 17 |
| 0.0 | 210,569 | 305,048 | 279,556 | 500,634 | 611,542 |
| 2.5 | 121,082 | 106,815 | 108,882 | 201,676 | 195,356 |
| 5.0 | 223,975 | 185,579 | 108,114 | 175,196 | 173,881 |
| 10.0 | 167,425 | 113,631 | 125,131 | 131,649 | 129,364 |
| 20.0 | 204,879 | 80,399 | 59,458 | 78,277 | 72,179 |
| 40.0 | 133,768 | 54,905 | 31,606 | 72,580 | 53,493 |

Addition of OvIFNτ produced a rapid, dose-dependent
30  decrease in reverse transcriptase (RT) activity (Table 4). While concentrations as low as 0.62 ng/ml of IFNτ inhibited viral replication, much higher concentrations (40 ng/ml) having greater effects on RT-activity were without toxic effects on the cells. The results suggest
35  that replication of the feline immunodeficiency virus was

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reduced significantly compared to control values when cells were cultured in the presence of OvIFNτ.

IFNτ appeared to exert no cytotoxic effect on the cells hosting the retrovirus. This was true even when
5   IFNτ was present at 40 ng per ml of culture medium.


## EXAMPLE 12
### Effects of IFNτ on HIV Infected
### Human Peripheral Lymphocytes

10   IFNτ was also tested for activity against HIV infection in human cells. Human peripheral blood lymphocytes, which had been infected with HIV (Crowe, et al.), were treated with varying concentrations of OvIFNτ. Replication of HIV in peripheral blood lymphocytes was monitored
15   by reverse transcriptase activity in culture supernatants over time. Reverse transcriptase activity was measured essentially by the method of Hoffman, et al. The data from these assays are presented in Table 5.


20                                   Table 5

### Effect of OvIFNτ on HIV Replication in
### Human Peripheral Lymphocytes

| IFNτ Concentration (ng/ml) | RT Activity | | | |
|---|---|---|---|---|
| | Day 6 | | Day 10 | |
| | cpm/ml | % Reduction | cpm/ml | % Reduction |
| 0 | 4,214 | -- | 25,994 | -- |
| 10 | 2,046 | 51 | 9,883 | 62 |
| 50 | 1,794 | 57 | 4,962 | 81 |
| 100 | 1,770 | 58 | 3,012 | 88 |
| 500 | 1,686 | 60 | 2,670 | 90 |
| 1000 | 1,499 | 64 | 2,971 | 89 |

35
As shown in Table 5, concentrations of OvIFNτ produced significant antiviral effects. A concentration of only 10 ng/ml resulted in over a 50% reduction in RT

activity after only six days. A concentration of 500
ng/ml resulted in a 90% reduction in RT activity within
10 days.

The viability of human peripheral blood lymphocytes
after treatment with IFNτ, over a range of concentrations
for 3-13 days, was evaluated by trypan blue exclusion.
The results of this viability analysis are presented in
Table 6.

### Table 6

### Effect of OvIFNτ on Viability of HIV Infected Human Peripheral Lymphocytes

| IFNτ Concentration (ng/ml) | Viable Cells/ml x $10^5$ | | |
|---|---|---|---|
| | Day 3 | Day 6 | Day 13 |
| 0 | 16.0 | 7.5 | 5.3 |
| 10 | 13.0 | 7.5 | 6.0 |
| 50 | 13.0 | 11.5 | 9.0 |
| 100 | 15.0 | 8.5 | 9.5 |
| 500 | 16.5 | 12.0 | 11.0 |
| 1000 | 21.9 | 9.5 | 8.5 |

The data presented in Table 6 show no evidence of
cytotoxic effects attributable to the administration of
IFNτ.

### EXAMPLE 13

### Inhibition of Cellular Growth

The effects of IFNτ on cellular growth were also
examined. Anti-cellular growth activity was examined
using a colony inhibition assay. Human amnion (WISH) or
MDBK cells were plated at low cell densities to form
colonies originating from single cells. Cells were
cultured at 200 or 400 cells/well in 24 well plates in
HMEM supplemented with 2% fetal bovine serum (FBS) and

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essential and nonessential amino acids.  Various dilu-
tions of interferons were added to triplicate wells, and
the plates were incubated for 8 days to allow colony
formation.  Colonies were visualized after staining with
5  crystal violet, and counted.  Cell cycle analysis was
performed with HMEM containing 0.5% "spent" media for an
additional 7 days.  WISH cells were used without being
synchronized.

For examination of IFNτ activity, cells were re-
10  plated at 2.5 × $10^5$ cells/well in HMEM with 10% FBS in 6
well plates.  Various dilutions of IFNτ alone or in
combination with peptides were added to achieve a final
volume of 1 ml.  Plates were incubated at 37°C in 5% $CO_2$
for 12, 15, 18, 24, or 48 hours.  Cells were treated with
15  trypsin, collected by low speed centrifugation and
washed.  The cell pellet was blotted dry and 250 μl of
nuclear staining solution (5 mg propidium iodide, 0.3 ml
NP40 and 0.1 gm sodium citrate i 100 ml distilled $H_2O$) was
added to each tube.  The tubes were incubated at room
20  temperature.  After 10 minutes, 250 μl of RNase (500
units/ml in 1.12% sodium citrate) was added per tube and
incubated an additional 20 minutes.  Nuclei were filtered
through 44 μm mesh, and analyzed on a FACStar (Becton
Dickinson, Mountain View, CA) using the DNA Star 2.0
25  software.

In the cellular growth assay using colony formation
of both the bovine epithelial line, MDBK, and the human
amniotic line, WISH, OvIFNτ inhibited both colony size
and number.  Ovine IFNτ was more effective than human
30  IFNα on the human cell line; thus, it is very potent in
cross-species activity.  Its activity was dose-dependent,
and inhibition of proliferation could be observed at
concentrations as low as 1 unit/ml.  Concentrations as
high as 50,000 units/ml (units of antiviral activity/ml)

stopped proliferation, while cell viability was not
impaired.

Cell cycle analysis by flow cytometry with propidium
iodide-stained WISH cells revealed an increased propor-
tion of cells in G2/M after 48 hours of OvIFNτ treatment.
IFNτ, therefore, appears to inhibit progress of cells
through S phase.  Ovine IFNτ antiproliferative effects
can be observed as early as 12 hours after the initiation
of culture and are maintained through 6 days.

The results presented above demonstrate both the
antiproliferative effect of IFNτ as well as its low
cytotoxicity.


EXAMPLE 14
Further Antiproliferative Effects of IFNτ

The antiproliferative effects of OvIFNτ were studied
for a rat cell line and a bovine cell line.  The rate of
$^3$H-thymidine incorporation was used to assess the rate of
cellular proliferation.

Rat (MtBr7 .c5) or bovine kidney (MDBK) cells were
seeded in phenol red-free DME-F12 medium supplemented
with 3% dextran-coated charcoal stripped Controlled
Process Serum Replacement 2 (CPSR 2, Sigma) and 5% dex-
tran-coated charcoal stripped fetal bovine serum (FBS).
After attaching for approximately 15-18 hours, the cells
were washed once with serum-free DME-F12 medium.  The
medium was replaced with phenol red-free DME-F12 medium
supplemented with 3% stripped CPSR2, 1% stripped FBS
("3/1" medium) or 3/1 medium containing OvIFNτ at various
units of antiviral activity as determined in the vesicu-
lar stomatitis virus challenge assay for interferons
(Example 2).  Media containing a similar dilution of
buffer (undiluted buffer = 10 mM Tris, 330 mM NaCl,

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[TS]), in which the OvIFNτ was dissolved was used for controls.

Cells were pulse labeled with $^3$H-thymidine for 2 hours at approximately 48 hours post-treatment. The trichloroacetic acid (TCA) precipitable incorporated counts were determined by scintillation counting. Three replicates were included per treatment. Mean values for OvIFNτ treatments were compared to samples containing comparable dilutions of carrier TS buffer. Results of these experiments are shown in Table 7.

## Table 7

### $^3$H-Thymidine Incorporation

| Treatment | % Reduction $^3$H-Thymidine Incorporation |
|---|---|
| Experiment 1: MtBr7 .c5 (Rat) | |
| 3/1 | - |
| $10^3$ u OvIFNτ/ml | 0 (+12) |
| 1:5000 TS | - |
| $10^4$ u OvIFNτ/ml | 24 |
| 1:500 TS | - |
| $10^5$ u OvIFNτ/ml | 24 |
| Experiment 2: MDBK | |
| 3/1 | - |
| $10^3$ u OvIFNτ/ml | 74 |
| 1:5000 TS | - |
| $10^4$ u OvIFNτ/ml | 83 |
| 1:500 TS | - |
| $10^5$ u OvIFNτ/ml | 83 |

As can be seen from Table 7, OvIFNτ drastically reduced the rate of cellular proliferation (based on thymidine incorporation) for each of the cell lines tested.

## EXAMPLE 15
### Antiproliferative Effects of IFNτ
### on Human Tumor Cell Lines

The antiproliferative activity of OvIFNτ on human

5   tumor cell lines was evaluated by measuring the rate of

$^3$H-thymidine incorporation into cells which have been

treated with OvIFNτ.

For experiments on tumor lines that grow in suspen-

sion, 1 ml of cells were plated at from 2.5 - 5 X $10^5$

10   cells/well in 24-well plates. Triplicate wells received

either the appropriate media, 100, 1,000 or 10,000

units/ml of OvIFNτ or equivalent antiviral concentrations

of rHuIFNα2A (Lee Biomolecular). After 48 hours of

incubation, cells were counted and viability assessed by

15   trypan blue exclusion.

Adherent tumor lines were plated at 2.5 X $10^5$

cells/well in 1 ml in 6-well plates. They received

interferon treatments as just described, but were tryp-

sinized prior to counting.

20   Significant differences between treatments were

assessed by an analysis of variance followed by Scheffe's

F-test. Cell cycle analysis was performed by flow cyto-

metry using propidium iodide.

25   A.   Breast Adenocarcinoma Cells.

Human MCF7 breast adenocarcinoma cells were seeded

from logarithmically growing cultures in phenol red-free

DME-F12 medium supplemented with 3% dextran-coated char-

coal stripped CPSR and 5% dextran-coated FBS. After

30   attaching for approximately 15-18 hours, the cells were

washed once with serum-free DME-F12 medium. The medium

was replaced with phenol red-free DME-F12 medium supple-

mented with 3% stripped CPSR2, 1% stripped FBS ("3/1"

medium) or 3/1 medium containing OvIFNτ at the indicated

35   number of units of antiviral activity as determined in

70

the vesicular stomatitis virus challenge assay for inter-
ferons.  Media containing a similar dilution of buffer
(undiluted buffer = 10 mM Tris, 330 mM NaCl [TS]) was
used for controls.  Cells were pulse labeled with $^3$H-
5    thymidine for 2 hours at approximately 48 hours post-
treatment.

The trichloroacetic acid (TCA) precipitable incorpo-
rated counts were determined by scintillation counting.
Three replicates were included per treatment.  Mean
10   values for OvIFNτ treatments were compared to samples
containing comparable dilutions of carrier TS buffer.
The results of these analyses are shown in Table 8.

### Table 8

15   ### $^3$H-Thymidine Incorporation

| Treatment | % Reduction $^3$H-Thymidine Incorporation |
|-----------|-------------------------------------------|
| MCF7  Human | |
| 3/1 | — |
| $10^3$ u OvIFNτ/ml | 35 |
| 1:5000 TS | — |
| $10^4$ u OvIFNτ/ml | 53 |
| 1:500 TS | — |
| $10^5$ u OvIFNτ/ml | 70 |

As can be seen from the results shown in Table 8,
OvIFNτ was able to substantially reduce the rate of $^3$H-
thymidine incorporation in the human carcinoma cell line.
30   This demonstrates the efficacy of OvIFNτ in inhibiting
tumor cell proliferation, in particular, mammary tumor
cell proliferation.

B.  Human Promyelocytic Leukemia.

A comparison of the antiproliferative effects of OvIFNτ and IFNα was conducted using HL-60 (human leukemia) cells (Foa, *et al.*; Todd, *et al.*) essentially as described above for MDBK cells.  Both OvIFNτ and rHuIFNα inhibit HL-60 cell proliferation.  Results of one of three replicate experiments are presented as mean % growth reduction ± SD in Figure 4.  Figure 4 shows that both OvIFNτ and IFNα were able to drastically reduce growth of HL-60 cells.  The growth reduction for each compound exceeded 60% for each concentration tested.  At 10,000 units/ml, OvIFNτ caused an approximately 80% reduction in growth while IFNα caused a 100% reduction in growth.

However, the data presented in Figure 4 reveal, that a substantial factor in the ability of IFNα to reduce growth was its toxic effect on the cells.  At 10,000 units/ml, the toxicity of IFNα resulted in less than 25% of the cells remaining viable.  By contrast, nearly 100% of the cells remained viable when OvIFNτ was applied at 10,000 units/ml.

Figure 5 presents data demonstrating that rHuIFNα is cytotoxic.  In the figure, results of one of three replicate experiments are presented as mean % viability ± SD.

C.  Human Cutaneous T Cell Lymphoma.

The cutaneous T cell lymphoma, HUT 78, responded similarly to HL-60 when treated with IFNτ (Figure 9).  Both OvIFNτ and rHuIFNα reduce HUT 78 cell growth, but 10,000 units/ml of rHuIFNα decreased the cell number below that originally plated ($5 \times 10^5$).  This is indicative of a reduction in cell viability to approximately 60%.

Cell cycle analysis (performed by cell flow cytometry) revealed an increased proportion of cells in G2/M

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phase of the cell cycle upon 48 hours of treatment with
both interferons (Table 10).  In Table 10 the results
from one of three replicate experiments are presented as
the percentage of cells in each phase of the cell cycle.
5    10,000 events were analyzed per sample.

This result is likely due to the slower progress of
cells through the cell cycle.  In the sample treated with
10,000 units/ml of rHuIFNα, a large percentage of events
with low forward and high side scatter, identifying dead
10   cells, were present.  This is consistent with the data
obtained from proliferation experiments, where only
OvIFNτ inhibited HUT 78 proliferation without toxicity.


Table 10.   HUT 78 Cell Cycle Analysis.

15

| Treatment (units/ml) | G0/G1 | S | G2/M |
|---|---|---|---|
| Media | 44.43 | 49.95 | 5.61 |
| 100 OvIFNτ | 44.35 | 47.45 | 8.20 |
| 100 rHuIFNα | 40.01 | 57.53 | 2.45 |
| 1,000 OvIFNτ | 41.29 | 50.50 | 8.21 |
| 1,000 rHuIFNα | 41.73 | 44.91 | 13.36 |
| 10,000 OvIFNτ | 42.79 | 42.61 | 14.60 |
| 10,000 rHuIFNα | 18.01 | 71.31 | 10.67 (cell death) |

25       D.  Human T Cell Lymphoma.
The T cell lymphoma cell line H9 was slightly less
sensitive to the antiproliferative effects of the IFNs
than the tumor cell lines described above.  Results of
one of three replicate experiments are presented in
30   Figure 10 as mean % growth reduction ± SD.  While rHuIFNα
was not toxic to the H9 cells, it failed to inhibit cell
division significantly at any of the concentrations
examined.  In contrast, OvIFNτ was observed to reduce H9
growth by approximately 60% (Figure 10).  Thus, only

OvIFNτ is an effective growth inhibitor of this T cell lymphoma.

The results presented above demonstrate both the antiproliferative effect of IFNτ as well as its low cytotoxicity.

## EXAMPLE 16
### Preliminary *In Vivo* Treatment with OvIFNτ

Three groups of 4 C57Bl/6 mice per group were given 2.5 X 10⁴ B16-F10 cells via the tail vein: B16-F10 is a syngeneic mouse transplantable tumor selected because of its high incidence of pulmonary metastases (Poste, *et al.*, 1981). Interferon treatment was initiated 3 days after the introduction of the tumor cells. Each mouse received 100 μl of either PBS alone, PBS containing 1 X 10⁵ units of OvIFNτ, or PBS containing 1 X 10⁵ units of recombinant murine IFNα (MuIFNα), i.v. per day for 3 consecutive days.

Mice were sacrificed at 21 days and the lungs were preserved in 10% buffered formalin. The frequency of pulmonary metastases were compared between control mice (PBS), OvIFNτ-treated mice, and MuIFNα-treated mice. The results of these *in vivo* administrations demonstrated that OvIFNτ dramatically reduced B16-F10 pulmonary tumors. These results support the use of IFNτ as an efficacious antineoplastic agent *in vivo*.

## EXAMPLE 17
### Competitive Binding of IFNτ Peptide Fragments
**A. The Ability of IFNτ-Based Peptides to Block IFNτ and IFN-α Antiviral Activity.**

Overlapping synthetic peptides were synthesized corresponding to the entire IFNτ sequence (Figure 6). Average hydropathicity values were calculated by taking

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the sum of the hydropathy values for each amino acid
divided by the total number of amino acids in each se-
quence.  Hydropathy values were taken from Kyte, *et al.*
(1982).

5      These peptides were of approximately the same molec-
ular weight but differed slightly in overall hydrophili-
city.  Despite this difference, all peptides were anti-
genic as demonstrated by the production of rabbit anti-
sera with titers greater than 1:3,000 as assessed by

10     ELISA (Harlow, *et al.*).

The peptides were used to inhibit the antiviral
activity (Example 2) of OvIFNτ and rBoIFNα.  The results
of this analysis are presented in Figure 12:  1 mM N- and
C-terminal peptides both effectively blocked the antivi-

15     ral activity of OvIFNτ using MDBK cells.  A third pep-
tide, representing amino acids 62-92, also reduced IFNτ
antiviral activity (70% inhibition).  The peptide OvIFNτ
(119-150) showed minimal inhibitory activity.  The OvIFNτ
(34-64) and (90-122) peptides had no apparent inhibitory

20     activity.

Peptide inhibition of OvIFNτ antiviral activity was
also examined as follows.  Monolayers of Madin Darby
bovine kidney cells were incubated with 40 units/ml
OvIFNτ in the presence or absence of various concentra-

25     tions of OvIFNτ peptides (see Figure 13).  Results in
Figure 13 are expressed as the percent of control antivi-
ral activity:  that is, in the absence of any competing
peptide.  Data presented are the means of 6 replicate
experiments.  The data demonstrate that inhibition by

30     OvIFNτ (1-37), (62-92), (119-150), and (139-172) were
significantly different than OvIFNτ (34-64) and (90-122)
at $10^{-3}$ M and 3 x $10^{-3}$ M.  OvIFNτ (139-172) was signifi-
cantly different than all other peptides at $10^{-3}$ M.  Sig-
nificance was assessed by analysis of variance followed

by Scheffe's F test at p < 0.05. Thus, OvIFNτ (1-37) (62-92), (119-150), and (139-172), in particular (139-172), may represent receptor binding regions for IFNτ.

The ability of the OvIFNτ peptides to inhibit bovine

5   IFNα (BoIFNα) antiviral activity was examined as follows. Monolayers of Madin Darby bovine kidney cells were incubated with 40 units/ml bovine IFNα in the presence or absence of various concentrations of OvIFNτ peptides. The results are presented in Figure 14 and are expressed

10  as the percent of control antiviral activity in the absence of OvIFNτ peptides. The data presented are the means of 4 replicate experiments. The results indicate that inhibition by OvIFNτ (62-92), (119-150), and (139-172) were significantly different from OvIFNτ (1-37),

15  (34-64) and (90-122) at $10^{-3}$ M. OvIFNτ (139-172) was significantly different than OvIFNτ (1-37), (34-64) and (90-122) at 3 x $10^{-3}$ M. Significance was assessed by analysis of variance followed by Scheffe's F test at p < 0.05. Thus, OvIFNτ (62-92), (119-150), and (139-172), in

20  particular (139-172), may represent common receptor binding regions for IFNτ and bovine IFNα.

Peptide inhibition by OvIFNτ peptides of human IFNα antiviral activity was also examined. Monolayers of Madin Darby bovine kidney cells were incubated with 40

25  units/ml human IFNα in the presence or absence of various concentrations of OvIFNτ peptides. The results are expressed as the percent of control antiviral activity in the absence of OvIFNτ peptides. The data are presented in Figure 15 and are the means of 3 replicate experi-

30  ments. OvIFNτ (139-172) was significantly different from all other peptides at $10^{-3}$ M. Significance was assessed by analysis of variance followed by Scheffe's F test at p < 0.05. Thus, OvIFNτ (139-172) may represent a common receptor binding region for IFNτ and various IFNα(s).

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The OvIFNτ peptides described above appear to have
no effect on the antiviral activity of IFNγ.  Peptide
inhibition of bovine IFNγ antiviral activity was evaluat-
ed as follows.  Monolayers of Madin Darby bovine kidney
5    cells were incubated with 40 units/ml bovine IFN gamma in
the presence or absence of various concentrations of
OvIFNτ peptides.  Results are expressed as the percent of
control antiviral activity in the absence of OvIFNτ
peptides.  The data are presented in Figure 16 and are
10   the means of 3 replicate experiments.  There were no
significant differences among peptides as assessed by
analysis of variance followed by Scheffe's F test at p <
0.05.

The two synthetic peptides OvIFNτ(1-37) and
15   OvIFNτ(139-172) also blocked OvIFNτ anti-FIV and anti-HIV
activity.  Reverse transcriptase (RT) activity (Examples
12 and 13) was monitored over a 14 day period in FIV-
infected FET-1 cells (1 X $10^6$/ml) and HIV-infected HPBL (1
X $10^6$/ml).  Control cultures received no OvIFNτ.  OvIFNτ
20   was used at 100 ng/ml, and peptides were used at 200 $\mu$M.
Data from a representative experiment are expressed as
cpm/ml culture supernatant and are presented for FIV
infected cells, Figure 11A, and HIV infected cells,
Figure 11B.  Both the N- and C-terminus of OvIFNτ appear
25   to be involved in its anti-retroviral activity.  While
both peptides blocked FIV RT activity, only the C-termi-
nal peptide, OvIFNτ(139-172), was an efficient inhibitor
of vesicular stomatitis virus activity on the feline cell
line, Fc9.  Thus the C-terminal regions of type I IFNs
30   may bind to common site on the type I IFN receptor, while
the N-terminal region may be involved in the elicitation
of unique functions.

B.  Anti-Peptide Sera.

The ability of anti-peptide antisera to inhibit OvIFNτ antiviral activity was also determined. Antipeptide antisera inhibition of OvIFNτ antiviral activity was
5    evaluated as follows. Monolayers of MDBK cells were incubated with 20 units/ml of OvIFNτ in the presence a 1:30 dilution of either preimmune sera or antisera to each of the OvIFNτ peptides described above. In Figure 17 the data from duplicate experiments are presented as
10   the mean percent inhibition of OvIFNτ antiviral activity produced by antipeptide antisera relative to the appropriate preimmune sera ± standard error. Significant differences were assessed by analysis of variance followed by Scheffe's F test at $p < 0.05$. Consistent with
15   peptide inhibition of antiviral activities, sera containing antibodies immunoreactive to OvIFNτ (1-37), OvIFNτ (62-92), and OvIFNτ (139-172) were also the most effective inhibitors of OvIFNτ antiviral activity, with antibodies directed against the N-terminal and C-terminal
20   peptides being the most efficacious.

The same sera were also used to examine their effect on the binding of IFNτ to its receptor.


25   The IFNτ binding assay was carried out as follows. Five μg of IFNτ was iodinated for 2 minutes with 500 μCi of Na$^{125}$I (15 mCi/μg; Amersham Corporation, Arlington Heights, IL) in 25 μl of 0.5 M potassium phosphate buffer, pH 7.4, and 10 μl of chloramine-T (5 mg/ml) (Griggs,
30   et al., 1992). The specific activity of the iodinated protein was 137 μCi/μg. For binding assays, monolayers of MDBK cells were fixed with paraformaldehyde and blocked with 5% nonfat dry milk. Cells were incubated with 5 nM $^{125}$I-IFNτ in phosphate buffered saline with 1%

BSA for 2 hours at 4°C in the presence or absence of a
1:30 dilution of sera containing antibodies raised
against IFNτ peptides or the appropriate preimmune sera.
Specific binding was assessed by incubation with a 100-
5    fold molar excess of unlabeled IFNτ.  Specific binding of
36% was determined by competition with 500 nM unlabeled
IFNτ.  For example, total counts bound were 6850 ± 133,
and a 100-fold molar excess of OvIFNτ produced 4398 ± 158
counts per minute.  After incubation, the monolayers were
10   washed three times, solubilized with 1% sodium dodecyl
sulfate, and the radioactivity counted.  Data from three
replicate experiments are presented in Figure 18 as the
mean percent reduction of OvIFNτ specific binding pro-
duced by antipeptide antisera relative to the appropriate
15   preimmune sera ± standard deviation.  Significant differ-
ences were assessed by analysis of variance followed by
Scheffe's F test.

The same sera (containing antibodies immunoreactive
to OvIFNτ (1-37), OvIFNτ (62-92), and OvIFNτ (139-172))
20   were the most effective inhibitors of $^{125}$I-IFNτ binding to
its receptor on MDBK cells.  The lack of effect of sera
immunoreactive with other IFNτ-derived peptides was not a
function of titer against OvIFNτ, since each sera had
equal or greater titer to their respective peptide rela-
25   tive to the three inhibiting sera:  similar results were
obtained when sera reactivity against the whole OvIFNτ
molecule was assessed by ELISA for each sera.

These peptides, although apparently binding to the
interferon receptor, did not in and of themselves elicit
30   interferon-like effects in the cells.

C.  Anti-Proliferative Activity.
Functionally important sites for the antiprolifera-
tive activity of IFNτ were also examined using synthetic

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peptides (Table 11). Cellular proliferation was assayed
as described above using MDBK cells. MDBK cells were
cultured at $5 \times 10^5$ cells/well in experiments 1 and 2 or
$10 \times 10^5$ cells in experiment 3 and treated with medium

5  alone, IFNτ at a concentration of 300 units/ml and pep-
tides at 1 mM for 48 hours. Duplicate wells were counted
in each of three replicate experiments. For statistical
analysis, data were normalized based on medium alone and
assessed by analysis of variance followed by Least Sig-

10  nificant Difference multiplate comparison test (p >
0.05).

## Table 11

### Peptide Inhibition of IFNτ Antiproliferative Activity

| Treatment | Experiment 1 | | Experiment 2 | | Experiment 3 | |
|---|---|---|---|---|---|---|
| | Cell Count | Via-bility | Cell Count | Via-bility | Cell Count | Via-bility |
| Medium alone | $9.8 \times 10^6$ | 99% | $13.0 \times 10^6$ | 96% | $27.3 \times 10^6$ | 97% |
| IFNτ | $5.0 \times 10^6$ | 98% | $5.6 \times 10^6$ | 97% | $8.3 \times 10^6$ | 97% |
| IFNτ+IFNτ(1-37) | $6.3 \times 10^6$ | 100% | $10.6 \times 10^6$ | 98% | $13.4 \times 10^6$ | 100% |
| IFNτ+IFNτ(34-64) | $5.3 \times 10^6$ | 96% | $6.9 \times 10^6$ | 95% | $16.0 \times 10^6$ | 98% |
| IFNτ+IFNτ(62-92) | $6.5 \times 10^6$ | 97% | $9.2 \times 10^6$ | 93% | $8.9 \times 10^6$ | 96% |
| IFNτ+IFNτ(90-122) | $5.9 \times 10^6$ | 100% | $11.0 \times 10^6$ | 97% | $19.6 \times 10^6$ | 96% |
| IFNτ+IFNτ(119-150) | $8.4 \times 10^6$ | 100% | $13.2 \times 10^5$ | 96% | $31.8 \times 10^6$ | 90% |
| IFNτ+IFNτ(139-172) | $5.1 \times 10^6$ | 100% | $12.7 \times 10^6$ | 98% | $18.9 \times 10^5$ | 98% |

When proliferation of MDBK cells was monitored over
a two-day period, cell number increased roughly 2-fold
with greater than 95% viability. Addition of 300

30  units/ml of OvIFNτ entirely eliminated cell proliferation
without a decrease in cell viability. Ovine IFNτ (119-
150) was the most effective inhibitor of IFNτ antiproli-
ferative activity.

Antisera to IFNτ (119-150), which inhibited binding

35  of OvIFNτ to receptor, also reversed the OvIFNτ antipro-

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liferative effect. Several other peptides, notably IFNτ
(139-172), reversed the OvIFNτ antiproliferative effect,
but to a lesser extent.

5

EXAMPLE 18

<u>Further Analysis</u>
<u>of the Cellular and Anti-Viral Effects of IFNτ</u>

A.    <u>HIV Anti-Viral Effects</u>.

10    The antiviral effects of IFNτ against HIV were
evaluated by treating human PBMC cells with various
amounts of either recombinant ovine IFNτ (r-OvIFNτ) or
recombinant human IFNα$_2$ at the time of infection with HIV.
Drug was present throughout the experiment.  At day 7 and
15    day 14, p24 production was determined (by ELISA (Wang, *et
al*., 1988, 1989) and compared to a zero drug control.
The results of this analysis are presented in Table 12.

<u>Table 12</u>

20

| Amounts of Drug Units/ml | | % Inhibition Day 7 | % Inhibition Day 14 |
|---|---|---|---|
| IFNα | IFNτ | | |
| 10 | | 58%, 48% | 91%, 91% |
| | 26 | 48%, 45% | 88%, 59% |
| 100 | | 68%, 74% | 94%, 91% |
| | 260 | 58%, 51% | 82%, 70% |
| 1,000 | | 89%, 86% | 97%, 93% |
| | 2,600 | 65%, 68% | 87%, 79% |
| 10,000 | | 90%, 86% | 99%, 99% |
| | 26,000 | 77%, 85% | 77%, 96% |
| | 260,000 | 85%, 84% | 96%, 86% |

The data from these experiments support the conclu-
30    sion that, at relatively low concentrations, IFNα and
IFNτ are effective in reducing the replication of HIV in
human lymphocytes.

B.  *In vitro Cytotoxicity Test in PBMC's*

Human PBMC's were seeded at $5 \times 10^5$ cells/ml.  Cells were stimulated at day 0 with 3 $\mu$g/ml PHA.  Cells were treated with recombinant human IFNα2A (at concentrations

5  of 10, 100, 1,000 and 10,000 units/ml) and IFNτ (at concentrations of 2.6, 26, 260, 2,600, 26,000, 260,000, and 2,600,000 units/ml) in 200 $\mu$l/wells (4 replicates of each concentration using 96 well flat bottom plates). Control cultures were given no interferons.  After 4 days

10  of incubation, cells were pulsed for 9 hours using $^3$H-thymidine at 1 uCi/well.  The cells were harvested and the incorporation of labeled thymidine into DNA was determined (Figure 8).

No cytotoxicity was observed by measuring the uptake

15  of thymidine at any concentration of IFNτ.  However, rHuIFNα2 was toxic to cells at 1,000 units/ml.

In a second experiment, the same human PBMC's were treated with either IFNτ or human IFNα2A at concentrations of 100 units/ml or 10,000 units/ml.  After 3 days

20  or 8 days of incubation, viable cells were counted by flow cytometry.  The results of this analysis are presented in Table 13.

TABLE 13

| Treatment (units/ml) | Number of Viable Cells x 10,000 | |
|---|---|---|
| | Day 3 | Day 8 |
| No Treatment | 735 | 840 |
| INFτ 100 units/ml<br>IFNτ 10,000 units/ml | 745<br>695 | 860<br>910 |
| IFNα 100 units/ml<br>IFNα 10,000 units/ml | 635<br>680 | 750<br>495 |

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No cytotoxicity was observed in the cells treated
with IFNτ.  However, there was 10% cell death in IFNα
treated cells at Day 3 and 49% cell death at Day 8.


5          C.   Inhibition of Hepatitis B Virus DNA Replication
                in Hepatocytes
           The cell line used, HepG2-T14, is a human cell that
was derived from liver cells transfected with Hepatitis B
Virus (HBV).  The cell line semi-stably produces HBV
10   virus:  over time the cell line's production of HBV
intracellular DNA and secreted virus decreases.  In order
to maximize production of HBV DNA and virus, the cells
are pre-treated with deAZA-C (5-azacytidine; Miyoshi, et
al.) to induce production of the virus.  Treatment was
15   for 2-3 days and the amount of induction was about a
factor of two.
           The cells were then treated with either the IFNα and
IFNτ at levels of 0, 5,000, 10,000, 20,000 and 40,000
units per ml.
20         All levels of either IFNα or IFNτ reduced DNA pro-
duction by about a factor of 2 compared to the no drug
control.


           D.   Inhibition of Hepatospecific Messenger RNA
25              Production in Hepatocytes
           The hepatocyte cell line HepG2-T14 (described above)
was examined for the effects of IFNα and IFNτ on hepato-
specific mRNA production.  Cells were incubated in con-
centrations of IFNα or IFNτ at 0, 5,000, 10,000, 20,000,
30   and 40,000 units per ml.  The messenger RNAs for the
hepatocyte specific proteins Apo E and Apo A1 were de-
tected by hybridization analysis (Sambrook, et al.;
Maniatis, et al.) using probes specific for these two
mRNA's (Shoulders, et al., and Wallis, et al.).

No reduction of mRNA production was seen for Apo E or Apo Al mRNA production with up to 40,000 units of either IFNα or IFNτ. This result suggests that the reduction of viral DNA replication in previous experiments was not due to the effects of IFNs on cellular house-keeping activities; rather the reduction was likely due to specific inhibition of viral replication in the host cells.

## EXAMPLE 19

### Isolation of Interferon-τ Fusion Protein

Sepharose 4B beads conjugated with anti-beta galactosidase is purchased from Promega. The beads are packed in 2 ml column and washed successively with phosphate-buffered saline with 0.02% sodium azide and 10 ml TX buffer (10 mM Tris buffer, pH 7.4, 1% aprotinin).

The IFNτ coding sequence (e.g., Figure 7) is cloned into the polylinker site of lambda gt11. The IFNτ coding sequence is placed in-frame with the amino terminal β-galactosidase coding sequences in lambda gt11. Lysogens infected with gt11/IFNτ are used to inoculate 500 ml of NZYDT broth. The culture is incubated at 32°C with aeration to an O.D. of about 0.2 to 0.4, then brought to 43°C quickly in a 43°C water bath for 15 minutes to induce gt11 peptide synthesis, and incubated further at 37°C for 1 hour. The cells are pelleted by centrifugation, suspended in 10 ml of lysis buffer (10 mM Tris, pH 7.4 containing 2% "TRITON X-100" and 1% aprotinin added just before use.

The resuspended cells are frozen in liquid nitrogen then thawed, resulting in substantially complete cell lysis. The lysate is treated with DNaseI to digest bacterial and phage DNA, as evidenced by a gradual loss

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of viscosity in the lysate.  Non-solubilized material is
removed by centrifugation.

The clarified lysate material is loaded on the
Sepharose column, the ends of the column closed, and the
5     column placed on a rotary shaker for 2 hrs. at room
temperature and 16 hours at 4°C.  After the column set-
tles, it is washed with 10 ml of TX buffer.  The fused
protein is eluted with 0.1 M carbonate/bicarbonate buff-
er, pH10.  Typically, 14 ml of the elution buffer is
10    passed through the column, and the fusion protein is
eluted in the first 4-6 ml of eluate.

The eluate containing the fusion protein is concen-
trated in "CENTRICON-30" cartridges (Amicon, Danvers,
Mass.).  The final protein concentrate is resuspended in,
15    for example, 400 µl PBS buffer.  Protein purity is ana-
lyzed by SDS-PAGE.

For polyclonal antibodies, the purified fused pro-
tein is injected subcutaneously in Freund's adjuvant in a
rabbit.  Approximately 1 mg of fused protein is injected
20    at days 0 and 21, and rabbit serum is typically collected
at 6 and 8 weeks.


EXAMPLE 20
Preparation of Anti-IFNτ Antibody
25    A.    Expression of Glutathione-S-Transferase Fusion
             Proteins.

The IFNτ coding sequence (e.g., Figure 7) is cloned
into the pGEX vector (Boyer, et al.; Frangioni, et al.;
Guan, et al.; Hakes, et al.; Smith, et al., 1988).  The
30    pGEX vector (Smith, et al.) was modified by insertion of
a thrombin cleavage sequence in-frame with the glutathi-
one-S-transferase protein (GST -- sj26 coding sequence).
This vector is designated pGEXthr.  The IFNτ coding
sequence is placed in-frame with the sj26-thrombin coding
35    sequences (Guan, et al.; Hakes, et al.).  The IFNτ coding

sequence insert can be generated by the polymerase chain reaction using PCR primers specific for the insert.

The IFNτ fragment is ligated to the linearized pGEXthr vector. The ligation mixture is transformed into
5    E. coli and ampicillin resistant colonies are selected. Plasmids are isolated from the ampicillin resistant colonies and analyzed by restriction enzyme digestion to identify clones containing the IFNτ insert (vector desig- nated pGEXthr-IFNτ).
10        E. coli strain XL-I Blue is transformed with pGEXthr-IFNτ and is grown at 37°C overnight. DNA is prepared from randomly-picked colonies. The presence of the insert coding sequence is typically confirmed by (i) restriction digest mapping, (ii) hybridization screening
15    using labelled IFNτ probes (i.e., Southern analysis), or (iii) direct DNA sequence analysis.


B.   Partial Purification of Fusion Proteins.

A pGEXthr-IFNτ clone is grown overnight. The over-
20   night culture is diluted 1:10 with LB medium containing ampicillin and grown for one hour at 37°C. Alternative- ly, the overnight culture is diluted 1:100 and grown to OD of 0.5-1.0 before addition of IPTG (isopropylthio-$\beta$- galactoside). IPTG (GIBCO-BRL, Gaithersburg MD) is added
25   to a final concentration of 0.2-0.5 mM for the induction of protein expression and the incubation is typically continued for 2-5 hours, preferably 3.5 hours.

Bacterial cells are harvested by centrifugation and resuspended in 1/100 culture volume of MTPBS (150 mM
30   NaCl, 16 mM $Na_2HPO_4$, 4 mM $NaH_2PO_4$). Cells are lysed by lysozyme, sonication or French press, and lysates cleared of cellular debris by centrifugation.

An aliquot of the supernatant obtained from IPTG- induced cultures of pGEXthr-IFNτ-containing cells and an

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aliquot of the supernatant obtained from IPTG-induced
cultures of pGEXthr-vector alone are analyzed by SDS-
polyacrylamide gel electrophoresis followed by Western
blotting, as described below.

5      If necessary, the extracts can be concentrated by
ultrafiltration using, for example, a "CENTRICON 10"
filter.

Alternatively, the fusion proteins are partially
purified over a glutathione agarose affinity column as

10     described in detail by Smith, et al.  In this method, 100
ml cultures are grown overnight.  The cultures are dilut-
ed to 1 liter, and the cells grown another hour at 37°C.
Expression of the fusion proteins is induced using IPTG.
The induced cultures are grown at 37°C for 3.5 hours.

15     Cells are harvested and a sonicator used to lyse the
cells.  Cellular debris is pelleted and the clear lysate
loaded onto a glutathione "SEPHAROSE" column.  The column
is washed with several column volumes.  The fusion pro-
tein is eluted from the affinity column with reduced

20     glutathione and dialyzed.  The IFNτ can be liberated from
the hybrid protein by treatment with thrombin.  The sj26
and IFNτ fragments of the hybrid protein can then be
separated by size fractionation over columns or on gels.

Alternatively, the IFNτ portion of the hybrid pro-

25     tein is released from the column by treatment with throm-
bin (Guan, et al.; Hakes, et al.).


C.   Antibodies Against the Fusion Protein.

The purified Sj26/IFNτ fused protein is injected

30     subcutaneously in Freund's adjuvant in a rabbit.  Approx-
imately 1 mg of fused protein is injected at days 0 and
21, and rabbit serum is typically collected at 6 and 8
weeks.  A second rabbit is similarly immunized with
purified Sj26 protein obtained from control bacterial

35     lysate.

Minilysates from the following bacterial cultures are prepared: (1) KM392 cells infected with pGEXthr and pGEXthr containing the IFNτ insert; and (2) cells infected with lambda gt11 containing the IFNτ insert. The

5 minilysates and a commercial source β-galactosidase are fractionated by SDS-PAGE, and the bands transferred to nitrocellulose filters for Western blotting (Sambrook, *et al.*; Ausubel, *et al.*).

Summarizing the expected results, serum from control

10 (Sj26) rabbits is immunoreactive with each of the Sj26 and Sj26 fused protein antigens. Serum from the animal immunized with Sj26/IFNτ fused protein is reactive with all Sj-26 and beta-gal fusion proteins containing IFNτ coding sequences, indicating the presence of specific

15 immunoreaction with the IFNτ antigen. None of the sera are expected to be immunoreactive with beta-galactosidase.

Anti-IFNτ antibody present in the sera from the animal immunized with the Sj26/IFNτ is purified by affinity

20 chromatography (using immobilized recombinantly produced IFNτ as ligand, essentially as described above in Example 12 for the anti-beta-galactosidase antibody).

While the invention has been described with refer-

25 ence to specific methods and embodiments, it will be appreciated that various modifications and changes may be made without departing from the invention.

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SEQUENCE LISTING

(1) GENERAL INFORMATION:

5          (i) APPLICANT: University of Florida
                         The Women's Research Institute


          (ii) TITLE OF INVENTION: Interferon Tau Compositions and
                                   Methods of Use
10
          (iii) NUMBER OF SEQUENCES: 20


          (iv) CORRESPONDENCE ADDRESS:
               (A) ADDRESSEE: Law Offices of Peter J. Dehlinger
15             (B) STREET: 350 Cambridge Ave., Suite 300
               (C) CITY: Palo Alto
               (D) STATE: CA
               (E) COUNTRY: USA
               (F) ZIP: 94306
20
          (v) COMPUTER READABLE FORM:
               (A) MEDIUM TYPE: Floppy disk
               (B) COMPUTER: IBM PC compatible
               (C) OPERATING SYSTEM: PC-DOS/MS-DOS
25             (D) SOFTWARE: PatentIn Release #1.0, Version #1.25


          (vii) PRIOR APPLICATION DATA:
               (A) APPLICATION NUMBER: US 07/969,890
               (B) FILING DATE: 30-OCT-1992
30
          (viii) ATTORNEY/AGENT INFORMATION:
               (A) NAME: Fabian, Gary R.
               (B) REGISTRATION NUMBER: 33,875
               (C) REFERENCE/DOCKET NUMBER: 5600-0001.41
35
          (ix) TELECOMMUNICATION INFORMATION:
               (A) TELEPHONE: 415-324-0880

(B) TELEFAX: 415-324-0960

(2) INFORMATION FOR SEQ ID NO:1:

5          (i) SEQUENCE CHARACTERISTICS:
               (A) LENGTH: 518 base pairs
               (B) TYPE: nucleic acid
               (C) STRANDEDNESS: double
               (D) TOPOLOGY: circular
10

          (ii) MOLECULE TYPE: DNA


          (iii) HYPOTHETICAL: NO


15        (iv) ANTI-SENSE: NO


          (vi) ORIGINAL SOURCE:
               (A) ORGANISM: Ovis aries
               (B) STRAIN: Domestic
20             (D) DEVELOPMENTAL STAGE: Blastula (blastocyst)
               (F) TISSUE TYPE: Trophectoderm
               (G) CELL TYPE: Mononuclear trophectoderm cells


          (vii) IMMEDIATE SOURCE:
25             (B) CLONE: oTP-1a


          (viii) POSITION IN GENOME:
               (C) UNITS: bp


30        (ix) FEATURE:
               (A) NAME/KEY: CDS
               (B) LOCATION: 1..518


          (x) PUBLICATION INFORMATION:
35             (A) AUTHORS: Ott, Troy L
                            Van Heeke, Gino
                            Johnson, Howard M
                            Bazer, Fuller W
               (B) TITLE: Cloning and Expression in Saccharomyces
40                  cerevisiae of a Synthetic Gene for the Type I
                    Trophoblast Interferon Ovine Trophoblast

90

Protein-1:Purification and Antiviral Activity

(C) JOURNAL: J. Interferon Res.

(D) VOLUME: 11

(F) PAGES: 357-364

5       (G) DATE: 1991

(K) RELEVANT RESIDUES IN SEQ ID NO:1: FROM 1 TO 518


(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

10

```
    TGC TAC CTG TCG CGA AAA CTG ATG CTG GAC GCT CGA GAA AAT TTA AAA          48
    Cys Tyr Leu Ser Arg Lys Leu Met Leu Asp Ala Arg Glu Asn Leu Lys
     1               5                  10                  15
```

```
15  CTG CTG GAC CGT ATG AAT CGA TTG TCT CCG CAC AGC TGC CTG CAA GAC          96
    Leu Leu Asp Arg Met Asn Arg Leu Ser Pro His Ser Cys Leu Gln Asp
                     20                  25 '               30
```

```
    CGG AAA GAC TTC GGT CTG CCG CAG GAA ATG GTT GAA GGT GAC CAA CTG         144
20  Arg Lys Asp Phe Gly Leu Pro Gln Glu Met Val Glu Gly Asp Gln Leu
                     35                  40                  45
```

```
    CAA AAA GAC CAA GCT TTC CCG GTA CTG TAT GAA ATG CTG CAG CAG TCT         192
    Gln Lys Asp Gln Ala Phe Pro Val Leu Tyr Glu Met Leu Gln Gln Ser
25       50                  55                  60
```

```
    TTC AAC CTG TTC TAC ACT GAA CAT TCT TCG GCC GCT TGG GAC ACT ACT         240
    Phe Asn Leu Phe Tyr Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr
     65                  70                  75                  80
30
    CTT CTA GAA CAA CTG TGC ACT GGT CTG CAA CAG CAA CTG GAC CAT CTG         288
    Leu Leu Glu Gln Leu Cys Thr Gly Leu Gln Gln Gln Leu Asp His Leu
                     85                  90                  95
```

```
35  GAC ACT TGC CGT GGC CAG GTT ATG GGT GAA GAA GAC TCT GAA CTG GGT         336
    Asp Thr Cys Arg Gly Gln Val Met Gly Glu Glu Asp Ser Glu Leu Gly
                     100                 105                 110
```

```
    AAC ATG GAT CCG ATC GTT ACT GTT AAA AAA TAT TTC CAG GGT ATC TAC         384
40  Asn Met Asp Pro Ile Val Thr Val Lys Lys Tyr Phe Gln Gly Ile Tyr
                     115                 120                 125
```

```
GAC TAC CTG CAG GAA AAA GGT TAC TCT GAC TGC GCT TGG GAA ATC GTA      432
Asp Tyr Leu Gln Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Ile Val
        130             135             140
```

```
5   CGC GTT GAA ATG ATG CGG GCC CTG ACT GTG TCG ACT ACT CTG CAA AAA      480
    Arg Val Glu Met Met Arg Ala Leu Thr Val Ser Thr Thr Leu Gln Lys
    145             150             155             160
```

```
    CGG TTA ACT AAA ATG GGT GGT GAC CTG AAT TCT CCG                      518
10  Arg Leu Thr Lys Met Gly Gly Asp Leu Asn Ser Pro
                    165             170
```

(2) INFORMATION FOR SEQ ID NO:2:

15

            (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 172 amino acids
                (B) TYPE: amino acid
                (D) TOPOLOGY: linear

20

        (ii) MOLECULE TYPE: protein


        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

25

    Cys Tyr Leu Ser Arg Lys Leu Met Leu Asp Ala Arg Glu Asn Leu Lys
    1               5               10              15


    Leu Leu Asp Arg Met Asn Arg Leu Ser Pro His Ser Cys Leu Gln Asp
30              20              25              30


    Arg Lys Asp Phe Gly Leu Pro Gln Glu Met Val Glu Gly Asp Gln Leu
                35              40              45


35  Gln Lys Asp Gln Ala Phe Pro Val Leu Tyr Glu Met Leu Gln Gln Ser
            50              55              60


    Phe Asn Leu Phe Tyr Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr
    65              70              75              80
40

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```
        Leu Leu Glu Gln Leu Cys Thr Gly Leu Gln Gln Gln Leu Asp His Leu
                         85                  90                  95

        Asp Thr Cys Arg Gly Gln Val Met Gly Glu Glu Asp Ser Glu Leu Gly
   5                    100                 105                 110

        Asn Met Asp Pro Ile Val Thr Val Lys Lys Tyr Phe Gln Gly Ile Tyr
                        115                 120                 125

  10    Asp Tyr Leu Gln Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Ile Val
                    130                 135                 140

        Arg Val Glu Met Met Arg Ala Leu Thr Val Ser Thr Thr Leu Gln Lys
        145                 150                 155                 160
  15
        Arg Leu Thr Lys Met Gly Gly Asp Leu Asn Ser Pro
                        165                 170
```

  20    (2) INFORMATION FOR SEQ ID NO:3:

            (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 516 base pairs
                (B) TYPE: nucleic acid
  25            (C) STRANDEDNESS: single
                (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: cDNA

  30        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

        TGTGACTTGT CTCAAAACCA CGTTTTGGTT GGTAGAAAGA ACTTAAGACT ACTAGACGAA        60

        ATGAGACGTC TATCTCCACA CTTCTGTCTA CAAGACAGAA AGGACTTCGC TTTGCCTCAG       120
  35
        GAAATGGTTG AAGGTGGCCA ACTACAAGAA GCTCAAGCGA TATCTGTTTT GCACGAAATG       180

        TTGCAACAAA GCTTCAACTT GTTCCACACC GAACACTCTT CGGCCGCTTG GGACACCACC       240

  40    TTGTTGGAAC CATGTAGAAC CGGTTTGCAC CAACAATTGG ACAACTTGGA TGCATGTTTG       300
```

GGTCAAGTTA TGGGTGAAGA AGACTCTGCT CTCGGGAGAA CCGGTCCAAC GCTAGCTTTG     36(

AAGAGATACT TCCAAGGTAT CCACGTTTAC TTGAAGGAAA AGGGTTACTC TGACTGTGCT     42(

TGGGAAACCG TGCGTCTAGA AATCATGCGT AGCTTCTCTT CTTTGATCAG CTTGCAAGAA     48(

AGATTACGTA TGATGGACGG TGACTTGTCG AGCCCA     51€


(2) INFORMATION FOR SEQ ID NO:4:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 172 amino acids
        (B) TYPE: amino acid
        (C) STRANDEDNESS: single
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: protein


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Cys Asp Leu Ser Gln Asn His Val Leu Val Gly Arg Lys Asn Leu Arg
1               5                   10                  15

Leu Leu Asp Glu Met Arg Arg Leu Ser Pro His Phe Cys Leu Gln Asp
            20                  25                  30

Arg Lys Asp Phe Ala Leu Pro Gln Glu Met Val Glu Gly Gly Gln Leu
        35                  40                  45

Gln Glu Ala Gln Ala Ile Ser Val Leu His Glu Met Leu Gln Gln Ser
        50                  55                  60

Phe Asn Leu Phe His Thr Glu His Ser Ser Ala Ala Trp Asp Thr Thr
65                  70                  75                  80

Leu Leu Glu Pro Cys Arg Thr Gly Leu His Gln Gln Leu Asp Asn Leu
                85                  90                  95

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```
         Asp Ala Cys Leu Gly Gln Val Met Gly Glu Glu Asp Ser Ala Leu Gly
                     100                 105                 110

         Arg Thr Gly Pro Thr Leu Ala Leu Lys Arg Tyr Phe Gln Gly Ile His
  5                  115                 120                 125

         Val Tyr Leu Lys Glu Lys Gly Tyr Ser Asp Cys Ala Trp Glu Thr Val
                     130                 135                 140

  10     Arg Leu Glu Ile Met Arg Ser Phe Ser Ser Leu Ile Ser Leu Gln Glu
                     145                 150                 155                 160

         Arg Leu Arg Met Met Asp Gly Asp Leu Ser Ser Pro
                         165                 170
  15
```

    (2) INFORMATION FOR SEQ ID NO:5:

            (i) SEQUENCE CHARACTERISTICS:
  20                (A) LENGTH: 37 amino acids
                    (B) TYPE: amino acid
                    (D) TOPOLOGY: linear

           (ii) MOLECULE TYPE: protein
  25
           (xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

```
         Cys Tyr Leu Ser Arg Lys Leu Met Leu Asp Ala Arg Glu Asn Leu Lys
                          5                 10                      15
  30
         Leu Leu Asp Arg Met Asn Arg Leu Ser Pro His Ser Cys Leu Gln Asp
                     20                 25                 30

         Arg Lys Asp Phe Gly
  35             35
```

    (2) INFORMATION FOR SEQ ID NO:6:

  40        (i) SEQUENCE CHARACTERISTICS:
                (A) LENGTH: 31 amino acids

(B) TYPE: amino acid

(D) TOPOLOGY: linear


(ii) MOLECULE TYPE: protein


(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:


Lys Asp Phe Gly Leu Pro Gln Glu Met Val Glu Gly Asp Gln Leu Gln
                  5                   10                  15


Lys Asp Gln Ala Phe Pro Val Leu Tyr Glu Met Leu Gln Gln Ser
              20                  25                  30



(2) INFORMATION FOR SEQ ID NO:7:


    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 31 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear


(ii) MOLECULE TYPE: protein


(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:


Gln Gln Ser Phe Asn Leu Phe Tyr Thr Glu His Ser Ser Ala Ala Trp
                  5                   10                  15


Asp Thr Thr Leu Leu Glu Gln Leu Cys Thr Gly Leu Gln Gln Gln
              20                  25                  30


(2) INFORMATION FOR SEQ ID NO:8:


    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 33 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear


(ii) MOLECULE TYPE: protein

96

                (xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

        Gln Gln Gln Leu Asp His Leu Asp Thr Cys Arg Gly Gln Val Met Gly
                         5                   10                  15

        Glu Glu Asp Ser Glu Leu Gly Asn Met Asp Pro Ile Val Thr Val Lys
                     20                  25                  30

        Lys


        (2) INFORMATION FOR SEQ ID NO:9:


                (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 32 amino acids
                    (B) TYPE: amino acid
                    (D) TOPOLOGY: linear


                (ii) MOLECULE TYPE: protein


                (xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

        Thr Val Lys Lys Tyr Phe Gln Gly Ile Tyr Asp Tyr Leu Gln Glu Lys
                         5                   10                  15

        Gly Tyr Ser Asp Cys Ala Trp Glu Ile Val Arg Val Glu Met Met Arg
                     20                  25                  30



        (2) INFORMATION FOR SEQ ID NO:10:


                (i) SEQUENCE CHARACTERISTICS:
                    (A) LENGTH: 34 amino acids
                    (B) TYPE: amino acid
                    (D) TOPOLOGY: linear


                (ii) MOLECULE TYPE: protein


                (xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

```
Cys Ala Trp Glu Ile Val Arg Val Glu Met Met Arg Ala Leu Thr Val
                    5                   10                  15
```

```
    Ser Thr Thr Leu Gln Lys Arg Leu Thr Lys Met Gly Gly Asp Leu Asn
5                   20                  25                  30
```

```
    Ser Pro
```

10  (2) INFORMATION FOR SEQ ID NO:11:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 588 base pairs
            (B) TYPE: nucleic acid
15          (C) STRANDEDNESS: double
            (D) TOPOLOGY: linear

        (ii) MOLECULE TYPE: DNA (genomic)

20      (iii) HYPOTHETICAL: NO

        (iv) ANTI-SENSE: NO

        (vi) ORIGINAL SOURCE:
25          (C) INDIVIDUAL ISOLATE: Human Interferon Tau Coding
                Sequences

        (ix) FEATURE:
            (A) NAME/KEY: CDS
30          (B) LOCATION: 1..585

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

```
35  ATG GCC TTC GTG CTC TCT CTA CTC ATG GCC CTG GTG CTG GTC AGC TAC        48
    Met Ala Phe Val Leu Ser Leu Leu Met Ala Leu Val Leu Val Ser Tyr
     1               5                   10                  15
```

```
    GGC CCA GGA GGA TCC CTG GGT TGT GAC CTG TCT CAG AAC CAC GTG CTG        96
40  Gly Pro Gly Gly Ser Leu Gly Cys Asp Leu Ser Gln Asn His Val Leu
                    20                  25                  30
```

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```
    GTT GGC AGG AAG AAC CTC AGG CTC CTG GAC GAA ATG AGG AGA CTC TCC        144
    Val Gly Arg Lys Asn Leu Arg Leu Leu Asp Glu Met Arg Arg Leu Ser
            35                  40                  45

 5  CCT CAC TTT TGT CTG CAG GAC AGA AAA GAC TTC GCT TTA CCC CAG GAA        192
    Pro His Phe Cys Leu Gln Asp Arg Lys Asp Phe Ala Leu Pro Gln Glu
            50                  55                  60

    ATG GTG GAG GGC GGC CAG CTC CAG GAG GCC CAG GCC ATC TCT GTG CTC        240
10  Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile Ser Val Leu
            65                  70                  75                  80

    CAT GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC CAC ACA GAG CAC TCC        288
    His Glu Met Leu Gln Gln Ser Phe Asn Leu Phe His Thr Glu His Ser
15                  85                  90                  95

    TCT GCT GCC TGG GAC ACC ACC CTC CTG GAG CCA TGC CGC ACT GGA CTC        336
    Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Pro Cys Arg Thr Gly Leu
                100                 105                 110
20

    CAT CAG CAG CTG GAC AAC CTG GAT GCC TGC CTG GGG CAG GTG ATG GGA        384
    His Gln Gln Leu Asp Asn Leu Asp Ala Cys Leu Gly Gln Val Met Gly
                115                 120                 125

25  GAG GAA GAC TCT GCC CTG GGA AGG ACG GGC CCC ACC CTG GCT CTG AAG        432
    Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu Ala Leu Lys
            130                 135                 140

    AGG TAC TTC CAG GGC ATC CAT GTC TAC CTG AAA GAG AAG GGA TAC AGC        480
30  Arg Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys Gly Tyr Ser
    145                 150                 155                 160

    GAC TGC GCC TGG GAA ACC GTC AGA CTG GAA ATC ATG AGA TCC TTC TCT        528
    Asp Cys Ala Trp Glu Thr Val Arg Leu Glu Ile Met Arg Ser Phe Ser
35                  165                 170                 175

    TCA TTA ATC AGC TTG CAA GAA AGG TTA AGA ATG ATG GAT GGA GAC CTG        576
    Ser Leu Ile Ser Leu Gln Glu Arg Leu Arg Met Met Asp Gly Asp Leu
                180                 185                 190
40
```

```
AGC TCA CCT TGA                                                    58
Ser Ser Pro
        195
```

(2)  INFORMATION FOR SEQ ID NO:12:

        (i)  SEQUENCE CHARACTERISTICS:
                (A)  LENGTH: 195 amino acids
                (B)  TYPE: amino acid
                (D)  TOPOLOGY: linear

        (ii)  MOLECULE TYPE: protein

        (xi)  SEQUENCE DESCRIPTION: SEQ ID NO:12:

```
Met Ala Phe Val Leu Ser Leu Leu Met Ala Leu Val Leu Val Ser Tyr
 1               5                   10                  15

Gly Pro Gly Gly Ser Leu Gly Cys Asp Leu Ser Gln Asn His Val Leu
            20                  25                  30

Val Gly Arg Lys Asn Leu Arg Leu Leu Asp Glu Met Arg Arg Leu Ser
        35                  40                  45

Pro His Phe Cys Leu Gln Asp Arg Lys Asp Phe Ala Leu Pro Gln Glu
    50                  55                  60

Met Val Glu Gly Gly Gln Leu Gln Glu Ala Gln Ala Ile Ser Val Leu
65                  70                  75                  80

His Glu Met Leu Gln Gln Ser Phe Asn Leu Phe His Thr Glu His Ser
                85                  90                  95

Ser Ala Ala Trp Asp Thr Thr Leu Leu Glu Pro Cys Arg Thr Gly Leu
                100                 105                 110

His Gln Gln Leu Asp Asn Leu Asp Ala Cys Leu Gly Gln Val Met Gly
            115                 120                 125
```

100

Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu Ala Leu Lys
    130                 135             140

Arg Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys Gly Tyr Ser
 5  145                 150             155             160

Asp Cys Ala Trp Glu Thr Val Arg Leu Glu Ile Met Arg Ser Phe Ser
                165             170             175

10  Ser Leu Ile Ser Leu Gln Glu Arg Leu Arg Met Met Asp Gly Asp Leu
                180             185             190

Ser Ser Pro
        195
15

(2) INFORMATION FOR SEQ ID NO:13:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 25 bases
20          (B) TYPE: nucleic acid
            (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear


        (ii) MOLECULE TYPE: DNA (synthetic)
25
        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:
    CCTGTCTGCA GGACAGAAAA GACTT                                          25



30  (2) INFORMATION FOR SEQ ID NO:14:

        (i) SEQUENCE CHARACTERISTICS:
            (A) LENGTH: 25 bases
            (B) TYPE: nucleic acid
35          (C) STRANDEDNESS: single
            (D) TOPOLOGY: linear


        (ii) MOLECULE TYPE: DNA (synthetic)

40      (xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:
    TCTGAATTCT GACGATTTCC CAGGC                                          25

(2) INFORMATION FOR SEQ ID NO:15:

   (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 37 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (iii) HYPOTHETICAL: NO

   (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment
                1-37, Human Tau-IFN

   (xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

   Cys Asp Leu Ser Gln Asn His Val Leu Val Gly Arg Lys Asn Leu Arg
   1               5                   10                  15

   Leu Leu Asp Glu Met Arg Arg Leu Ser Pro His Phe Cys Leu Gln Asp
                   20                  25                  30

   Arg Lys Asp Phe Ala
               35

(2) INFORMATION FOR SEQ ID NO:16:

   (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 31 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

   (ii) MOLECULE TYPE: peptide

   (iii) HYPOTHETICAL: NO

   (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment

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34-64, Human Tau-IFN


(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:


Lys Asp Phe Ala Leu Pro Gln Glu Met Val Glu Gly Gly Gln Leu Gln
1               5               10              15


Glu Ala Gln Ala Ile Ser Val Leu His Glu Met Leu Gln Gln Ser
                20              25              30


(2) INFORMATION FOR SEQ ID NO:17:


    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 31 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear


    (ii) MOLECULE TYPE: peptide


    (iii) HYPOTHETICAL: NO


    (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment
            62-92, Human Tau-IFN


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:


Gln Gln Ser Phe Asn Leu Phe His Thr Glu His Ser Ser Ala Ala Trp
1               5               10              15


Asp Thr Thr Leu Leu Glu Pro Cys Arg Thr Gly Leu His Gln Gln
                20              25              30

(2) INFORMATION FOR SEQ ID NO:18:


    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 33 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(iii) HYPOTHETICAL: NO

(vi) ORIGINAL SOURCE:
     (C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment
         90-122, Human Tau-IFN


(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

```
His Gln Gln Leu Asp Asn Leu Asp Ala Cys Leu Gly Gln Val Met Gly
1               5                   10                  15

Glu Glu Asp Ser Ala Leu Gly Arg Thr Gly Pro Thr Leu Ala Leu Lys
                20                  25                  30

Arg
```


(2) INFORMATION FOR SEQ ID NO:19:

    (i) SEQUENCE CHARACTERISTICS:
        (A) LENGTH: 32 amino acids
        (B) TYPE: amino acid
        (D) TOPOLOGY: linear

    (ii) MOLECULE TYPE: peptide

    (iii) HYPOTHETICAL: NO

    (vi) ORIGINAL SOURCE:
        (C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment
          119-150, Human Tau-IFN


    (xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

```
Ala Leu Lys Arg Tyr Phe Gln Gly Ile His Val Tyr Leu Lys Glu Lys
1               5                   10                  15
```

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```
Gly Tyr Ser Asp Cys Ala Trp Glu Thr Val Arg Leu Glu Ile Met Arg
            20                  25                  30
```

5   (2) INFORMATION FOR SEQ ID NO:20:

         (i) SEQUENCE CHARACTERISTICS:
             (A) LENGTH: 34 amino acids
             (B) TYPE: amino acid
10           (D) TOPOLOGY: linear


        (ii) MOLECULE TYPE: peptide


       (iii) HYPOTHETICAL: NO
15

        (vi) ORIGINAL SOURCE:
             (C) INDIVIDUAL ISOLATE: Amino acid sequence of fragment
                 139-172, Human Tau-IFN


20

        (xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:


         Cys Ala Trp Glu Thr Val Arg Leu Glu Ile Met Arg Ser Phe Ser Ser
         1               5                   10                  15
25

         Leu Ile Ser Leu Gln Glu Arg Leu Arg Met Met Asp Gly Asp Leu Ser
                     20                  25                  30


         Ser Pro
30
```

IT IS CLAIMED:

1.   A method of inhibiting tumor cell growth, comprising contacting the cells with interferonτ at a concentration effective to inhibit growth of the tumor cells.

2.   The method of claim 1, where said interferon-τ is that which can be obtained from a mammal of the group consisting of cows, sheep, and humans.

3.   The method of claim 1, wherein the interferon-τ comprises a sequence selected from the group consisting of  SEQ ID NO:2 and SEQ ID NO:4.

4.   The method of claim 1, wherein the interferon-τ has the sequence presented as SEQ ID NO:4.

5.   The method of claim 4, wherein said cells are human carcinoma cells, human leukemia cells, human T-lymphoma cells, and human melanoma cells.

6.   The method of claim 5, wherein said cells are steroid-sensitive tumor cells.

7.   The method of claim 6, wherein said cells are mammary tumor cells.

8.   A method of inhibiting viral replication in cells, comprising
    contacting cells infected with a virus with interferon-τ at a concentration effective to inhibit viral replication within said cells.

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9.   The method of claim 8, where said interferon-τ
is obtained from a mammal of the group consisting of
cows, sheep, and humans.

5        10.   The method of claim 8, wherein the interferon-τ
comprises a sequence selected from the group consisting
of:   SEQ ID NO:2 or SEQ ID NO:4.

11.   The method of claim 8, where said virus is an
10   RNA virus.

12.   The method of claim 11, where said virus is
selected from the group consisting of feline leukemia
virus, human immunodeficiency virus, or hepatitis c
15   virus.

13.   The method of claim 8, where said virus is
hepatitis B virus.

20        14.   A method of enhancing fertility in a female
mammal, comprising
administering to said mammal an effective mammalian
fertility enhancing amount of human interferon-τ in a
pharmaceutically acceptable carrier.
25

15.   The method of claim 14, where said interferon-τ
has the protein sequence presented as SEQ ID NO:4.

16.   An isolated nucleic acid which encodes a human
30   interferon-τ.

17.   The nucleic acid of claim 16, wherein said
nucleic acid molecule has the sequence presented as SEQ
ID NO:11.

35

18.  The nucleic acid of claim 16, wherein said nucleic acid molecule has the sequence presented as SEQ ID NO:3.

5       19.  An expression vector comprising

        (a)  a nucleic acid containing an open reading frame that encodes human interferon-τ; and

        (b)  regulatory sequences effective to express said open reading frame in a host cell.

10

        20.  The expression vector of claim 19, wherein said regulatory sequences include, 5' to said nucleic acid sequence, a promoter region and an ATG start codon in-frame with the interferon-τ coding sequence, and 3' to

15    said coding sequence, a translation termination signal followed by a transcription termination signal.

        21.  The expression vector of claim 19, wherein said nucleic acid is included in the sequence is selected from

20    the group consisting of SEQ ID NO:1, SEQ ID NO:3 and SEQ ID NO:11.

        22.  A recombinantly produced human interferon-τ protein.

25

        23.  The recombinantly produced protein of claim 22, which includes the sequence presented as SEQ ID NO:4.

        24.  The recombinantly produced protein of claim 23,

30    which further includes an amino terminal extension, where said protein has the sequence presented as SEQ ID NO:12.

        25.  A method of recombinantly producing interferon-τ, comprising

108

introducing into suitable host cells, a recombinant
expression system containing an open reading frame (ORF)
having a polynucleotide sequence which encodes a human
interferon-τ polypeptide, where the vector is designed to
5    express the ORF in said host, and
      culturing said host under conditions resulting in
the expression of the ORF sequence.


      26.   The method of claim 25, wherein the interferon-
10   τ polypeptide has the sequence presented as SEQ ID NO:4.


      27.   The method of claim 25, wherein the expression
vector is a lambda gt11 phage vector and the host cells
are *E. coli.*
15


      28.   The method of claim 25, wherein the polynucleo-
tide sequence has the sequence presented as SEQ ID NO:3.


      29.   The method of claim 28, wherein the host cells
20   are yeast.


      30.   The method of claim 28, wherein the host cells
are insect cells.


25        31.   The method of claim 25, wherein the polypeptide
has the sequence presented as SEQ ID NO:4, and the poly-
nucleotide has the sequence presented in SEQ ID NO:11.


      32.   An expression system for expressing a interfer-
30   on-τ polypeptide, comprising
      a host capable of supporting expression of an open
reading frame in a selected expression vector, and
      the selected expression vector containing an open
reading frame (ORF) having a polynucleotide sequence
35   which encodes a human interferon-τ polypeptide.

33. The expression system of claim 32, wherein the polypeptide is selected from the group consisting of: SEQ ID NO:4, SEQ ID NO:15, SEQ ID NO:16, SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19 and SEQ ID NO:20.

34. The expression system of claim 32, wherein the polypeptide is the polypeptide having the sequence presented as SEQ ID NO:4.

35. An isolated interferon-τ polypeptide, where said polypeptide is (i) derived from the interferon-τ amino acid coding sequence, and (ii) between 15 and 172 amino acids long.

36. The polypeptide of claim 35, where said interferon-τ sequence is selected from the group consisting of SEQ ID NO:2 and SEQ ID NO:4.

37. The polypeptides of claim 35, where said polypeptide is selected from the group consisting of SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:9 and SEQ ID NO:10.

38. The polypeptides of claim 35, where said polypeptide is selected from the group consisting of SEQ ID NO:15, SEQ ID NO:17, SEQ ID NO:19 and SEQ ID NO:20.

39. A method of blocking the binding of an alpha-interferon to a cell having an alpha-interferon receptor, comprising
contacting the cell with an interferon-τ polypeptide at a concentration effective to allow the binding of interferon-τ polypeptide to each alpha-interferon receptor, and exposing the cells having interferon-τ polypeptide bound to said receptor to alpha-interferon.

110

40. The method of claim 39, where the interferon-τ polypeptide is selected from the group consisting of SEQ ID NO:2, SEQ ID NO:5, SEQ ID NO:7 and SEQ ID NO:10.

5       41. The method of claim 39, where the interferon-τ polypeptide is selected from the group consisting of SEQ ID NO:4, SEQ ID NO:15, SEQ ID NO:17 and SEQ ID NO:20.

        42. A method of blocking the binding of interferon-
10   τ to a cell having a interferon-τ receptor, comprising contacting the cell with an interferon-τ polypeptide selected from the group consisting of SEQ ID NO:5, SEQ ID NO:7, SEQ ID NO:10, SEQ ID NO:15, SEQ ID NO:17 and SEQ ID NO:20, where said polypeptide is at a concentration
15   effective to allow the binding of the polypeptide to each interferon-τ-receptor, and exposing the cells having interferon-τ polypeptides bound to said receptor to interferon-τ.

20       43. Purified antibodies that are immunoreactive with human interferon-τ.

        44. The antibodies of claim 43 which are polyclonal antibodies.
25

        45. The antibodies of claim 43 which are monoclonal antibodies.

        46. The antibodies of claim 43, where said antibod-
30   ies are reactive with a polypeptide selected from the group consisting of SEQ ID NO:4, SEQ ID NO:15, SEQ ID NO:17 and SEQ ID NO:20.

        47. A fused polypeptide, comprising:

111

(a) an interferon-τ polypeptide, where said polypep-
tide is (i) derived from an interferon-τ amino acid
coding sequence, and (ii) between 15 and 172 amino acids
long; and

5          (b) a second soluble polypeptide.


48.   A fused polypeptide of claim 47, where said
interferon-τ sequence is selected from the group consist-
ing of SEQ ID NO:2 and SEQ ID NO:4.

10

49.   A fused polypeptide of claim 47, where said
polypeptide is selected from the group consisting of SEQ
ID NO:5, SEQ ID NO:7, SEQ ID NO:9 and SEQ ID NO:10.


15          50.   A fused polypeptides of claim 47, where said
polypeptide is selected from the group consisting of SEQ
ID NO:15, SEQ ID NO:17, SEQ ID NO:19 and SEQ ID NO:20.


51.   A fused polypeptide of claim 47, where said
20    second soluble polypeptide is serum albumin.


52.   A fused polypeptide of claim 47, where said
second soluble polypeptide is interferon-α.


25

# Fig. 1

METOTP-1.SEQ4 -> Restriction Map

DNA   sequence   540 b.p.   ATGTGGTACCAG ... CCGTAAGGTACC   linear

```
              Stu I
              Hae I
           ScrF I
           EcoR II
           BstN I
        Rsa I                              Taq I
        Nla IV                             Xho I
        Kpn I                              Pae27 I
        Ban I   Hae III      BstU I        Rca I       Mse I      Sau96 I    Cla I
        Asp718             Nru I    SfaN I   Ava I    Dra I      Ava II    Hinf I
        || | ||            ||       |        |  ||      ||         |        |.|
ATGTGGTACCAGGCCTGCTACCTGTCGCGAAAACTGATGCTGGACGCTCGAGAAAATTTAAAACTGCTGGACCGTATGAA  80
TACACCATGGTCCGGACGATGGACAGCGCTTTTGACTACGACCTGCGAGCTCTTTTAAATTTTGACGACCTGGCATACTT
        || |·||       ·    ||    ·    |   ·  |  ||·       ||·       |·       || |
        5    5         12        25       36       43       47        57        69        78
        5                        26                        47        58        69        80
        5                                                  47
        5                                                  47
        6                                                  48
            9
            9
            9
            11
            11

                          Fnu4H I
                          Bbv I
                          Alu I          Xmn I                                     Hae III
            BsmA I        Pvu II        Msp I                                      Bph I
Taq I                     NspB II       Hpa II              Fnu4H I                BstE II
|           |             | |||         | |                 |                      ||
TCGATTGTCTCCGCACAGCTGCCTGCAAGACCGGAAAGACTTCGGTCTGCCGCAGGAAATGGTTGAAGGTGACCAACTGC  160
AGCTAACAGAGGCGTGTCGACGGACGTTCTGGCCTTTCTGAAGCCAGACGGCGTCCTTTACCAACTTCCACTGGTTGACG
|           |    ·        | |||  ·       ·|   ·             |·          ||·
81                        96           111               129                148
     87                   96           111                               148
                          97                114                          149
                          98
                          98
                          Msp I
                          ScrF I                  Fnu4H I
                          Nci I                    Bbv I                            Fnu4H I
                          Bcn I                    Pst I                            Hae III
               Alu I   Hpa II                      Fnu4H I                          Gdi II
               HinD III    Rsa I                   Bbv I                            Eag I
               ||          || |                     |                              Eae I
                                                                         Mbo II    || |
AAAAAGACCAAGCTTTCCCGGTACTGTATGAAATGCTGCAGCAGTCTTTCAACCTGTTCTACACTGAACATTCTTCGGCC  240
TTTTTCTGGTTCGAAAGGGCCATGACATACTTTACGACGTCGTCAGAAAGTTGGACAAGATGTGACTTGTAAGAAGCCGG
               ||          || ·|                    || |                   ·       || |  ·
               170         181                      195                           232
               171         178                      195                           236
                          177                       196                           236
                          177                       198                           236
                          177                       198                           237
                          178                                                     238
                                                                                  ScrF I
                                                                                  PflM I
                                                                                  EcoR II
                                                                                  BstN I
                                                                                  Hae III
                                                                                  Hae I
                            Mae I           Bsr I                                  Eae I
                            Xba I           Hgia I               Sau96 I           Bal I
                          Mbo II            Bsp1286 I            Bsr I             Sec I
                          Ear I             ApaL I            AluV I   Ava II      DsaI
                          || ||              |    |             |     |   |        | ||| |
GCTTGGGACACTACTCTTCTAGAACAACTGTGCACTGGTCTGCAACAGCAACTGGACCATCTGGACACTTGCCGTGGCCA  320
CGAACCCTGTGATGAGAAGATCTTGTTGACACGTGACCAGACGTTGTCGTTGACCTGGTAGACCTGTGAACGGCACCGGT
                          · || ||·         |        ·  ·  |       ·| ·      ·  |  · || |  ·
                          254                270                286     294        312
                          255                270                     291           312
                          258                270                          294           315
                          259                274                                        315
                                                                                        315
```

# Fig. 1 (con't)

```
                                          Sau3A I
                                          Mbo I
                                    Sau3A I
                                    Mbo I
                                    Dpn I
                                    Alw I
                                    Nla IV
                         Ple I       BstY I
                        Mbo II       BamH I                   Sec I
                        Bbv II       Alw I      Mae III       ScrF I
              Mbo II                Mae III     Pvu I         EcoR II
        Hph I    Hinf I   Bsr I    Nla III   Dpn I    Mse I   Ssp I  BstN I
        | | |    |   |    |        |  ||     ||  |     |       |     |
        GGTTATGGGTGAAGAAGACTCTGAACTGGGTAACATGGATCCGATCGTTACTGTTAAAAAATATTTCCAGGGTATCTACG  400
        CCAATACCCACTTCTTCTGAGACTTGACCCATTGTACCTAGGCTAGCAATGACAATTTTTTTATAAAGGTCCCATAGATGC
        | •|    |   |    •        |  ||  • ||     |        |     |
        328      337      345      354      363        374        387
          331                     350       362                   387
            334                            357        367          387
            334                            357                     387
              337                          357
                                           357
                                           358
                                           358
                                           358
                                           358
                                           363
                                           363
```

```
                                                        Sau96 I
                                                        Hae III
                                                        Sau96 I
                                                        Nla IV
                                            BstU I       Eco0109 I    Taq I
                                            Mlu I        Bsp1286 I    Sal I
                                            Afl III      Ban II       HinC II
                Pst I                Hin P I    Rsa I     Apa I       Acc I
              BsrM I    Mae III     Hha I      Spl I    SfaN I  Tth111 I
              | |       |           |          || ||     |     ||         ||
        ACTACCTGCAGGAAAAAGGTTACTCTGACTGCGCTTGGGAAATCGTACGCGTTGAAATGATGCGGCCCCTGACTGTGTCG  480
        TGATGGACGTCCTTTTTCCAATGAGACTGACGCGAACCCTTTAGCATGCGCAACTTTACTACGCCGGGGACTGACACAGC
        | |   • •     |•         •|        •   || ||  •      |•    ||    •|      || •
        404          419         431       444       459      464    471
          406                    431       445                464           477
                                           447                464           477
                                           447                464           477
                                           448                464           478
                                                              464
                                                              464
                                                              465
                                                              465
```

```
                                                      Rsa I
                                                      Nla IV
                Mse I              Mae III            Kpn I
                Hpa I             Hph I    EcoR I     Ban I
                HinC II           BstE II             Asp718
                ||               ||        |          ||
        ACTACTCTGCAAAAACGGTTAACTAAAATGGGTGGTGACCTGAATTCTCGGTAAGGTACC  540
        TGATGAGACGTTTTTGCCAATTGATTTTACCCACCACTGGACTTAAGAGGCATTCCATGG
            •                ||•       ||    •|        ||   •
                498              514              535
                498              514       522    535
                499              515              535
                                                  535
                                                  536
```

Fig. 2
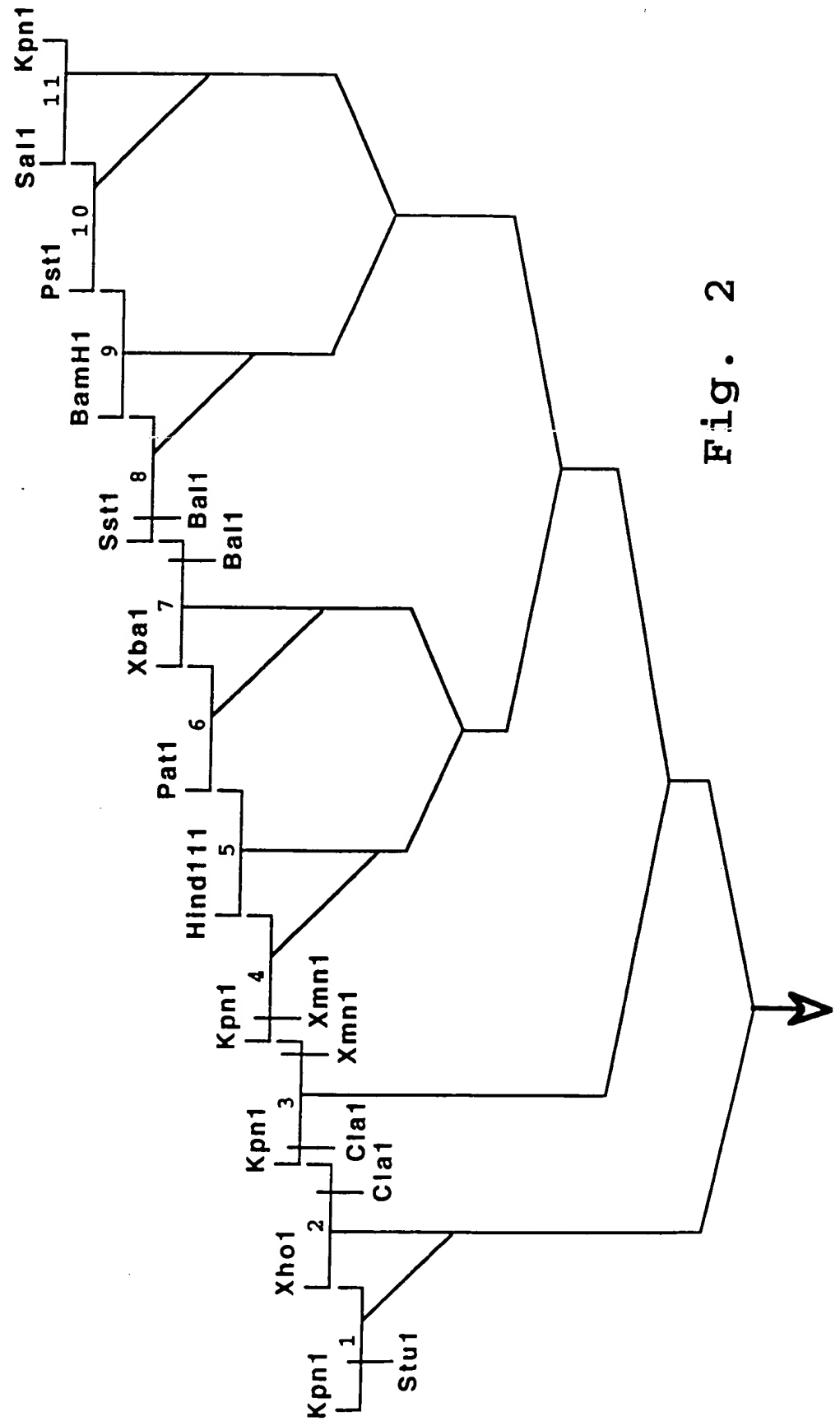
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                        met ala phe val leu ser leu leu met ala leu val leu val ser
                                                                                    -9
        oIFNτ   cccc ATG GCC TTC GTG CTC TCT CTA CTG ATG GCC CTG GTG CTG GTC AGC
        htIFN   cccc ATG GCC TTC GTG CTC TCT CTA CTC ATG GCC CTG GTG CTG GTC AGC

        -8                                      -1  +1
        tyr gly pro gly gly ser leu gly cys tyr leu ser glu arg leu met leu asp ala
                                                                                    11
        TAT GGC CCA GGA GGA TCT CTG GGT TGT TAC CTA TCT GAG AGA CTC ATG CTG GAT GCC
        TAC GGC CCA GGA GGA TCC CTG GGT TGT GAC CTG TCT CAG AAC CAC GTG CTG GTT GGC
                                              asp         gln asn his val     val gly

        12
        arg glu asn leu lys leu leu asp arg met asn arg leu ser pro his ser cys leu
                                                                                    30
        AGG GAG AAC CTC AAG CTC CTG GAC CGA ATG AAC AGA CTC TCC CCT.CAT TCC TGT CTG
        AGG AAG AAC CTC AGG CTC CTG GAC GAA ATG AGG AGA CTC TCC CCT CAC TTT TGT CTG
                lys             arg         glu     arg                 phe

        31
        gln asp arg lys asp phe gly leu pro gln glu met val glu gly asp gln leu gln
                                                                                    49
        CAG GAC AGA AAA GAC TTT GGT CTT CCC CAG GAG ATG GTG GAG GGC GAC CAG CTC CAG
        CAG GAC AGA AAA GAC TTC GCT TTA CCC CAG GAA ATG GTG GAG GGC GGC CAG CTC CAG
                                    ala                                 gly

        50
        lys asp gln ala phe pro val leu tyr glu met leu gln gln ser phe asn leu phe
                                                                                    68
        AAG GAC CAG GCC TTC CCT GTG CTC TAC GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC
        GAG GCC CAG GCC ATC TCT GTG CTC CAT GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC
        glu ala         ile ser         his

        69
        tyr thr glu his ser ser ala ala trp asp thr thr leu leu glu gln leu cys thr
                                                                                    87
        TAC ACA GAG CAC TCC TCT GCT GCC TGG GAC ACC ACC CTC CTG GAG CAG CTC TGC ACT
        CAC ACA GAG CAC TCC TCT GCT GCC TGG GAC ACC ACC CTC CTG GAG CCA TGC CGC ACT
        his                                                         pro cys arg

        88
        gly leu gln gln gln leu asp his leu asp thr cys arg gly gln val met gly glu
                                                                                    106
        GGA CTC CAA CAG CAG CTG GAC CAC CTG GAC ACC TGC AGG GGT CAA GTG ATG GGA GAG
        GGA CTC CAT CAG CAG CTG GAC AAC CTG GAT GCC TGC CTG GGG CAG GTG ATG GGA GAG
                his                     asn     ala     leu

        107
        glu asp ser glu leu gly asn met asp pro ile val thr val lys lys tyr phe gln
                                                                                    125
        GAA GAC TCT GAA CTG GGT AAC ATG GAC CCC ATT GTG ACC GTG AAG AAG TAC TTC CAG
        GAA GAC TCT GCC CTG GGA AGG ACG GGC CCC ACC CTG GCT CTG AAG AGG TAC TTC CAG
                        ala         arg thr gly     thr leu ala leu     arg

        126
        gly ile tyr asp tyr leu gln glu lys gly tyr ser asp cys ala trp glu ile val
                                                                                    144
        GGC ATC TAT GAC TAC CTG CAA GAG AAG GGA TAC AGC GAC TGC GCC TGG GAA ATC GTC
        GGC ATC CAT GTC TAC CTG AAA GAG AAG GGA TAC AGC GAC TGC GCC TGG GAA ACC GTC
                his val         lys                                         thr

        145
        arg val glu met met arg ala leu met val ser thr thr leu gln lys arg leu thr
                                                                                    163
        AGA GTC GAG ATG ATG AGA GCC CTC ACT GTA TCA ACC ACC TTG CAA AAA AGG TTA ACA
        AGA CTG GAA ATC ATG AGA TCC TTC TCT TCA TTA ATC AGC TTG CAA GAA AGG TTA AGA
            leu     ile         ser phe ser     ser leu ile ser     glu         arg

        164                             172
        lys met gly gly asp leu asn ser pro end
        AAG ATG GGT GGA GAT CTG AAC TCA CCT TGA
        ATG ATG GAT GGA GAC CTG AGC TCA CCT TGA
        met     asp         ser
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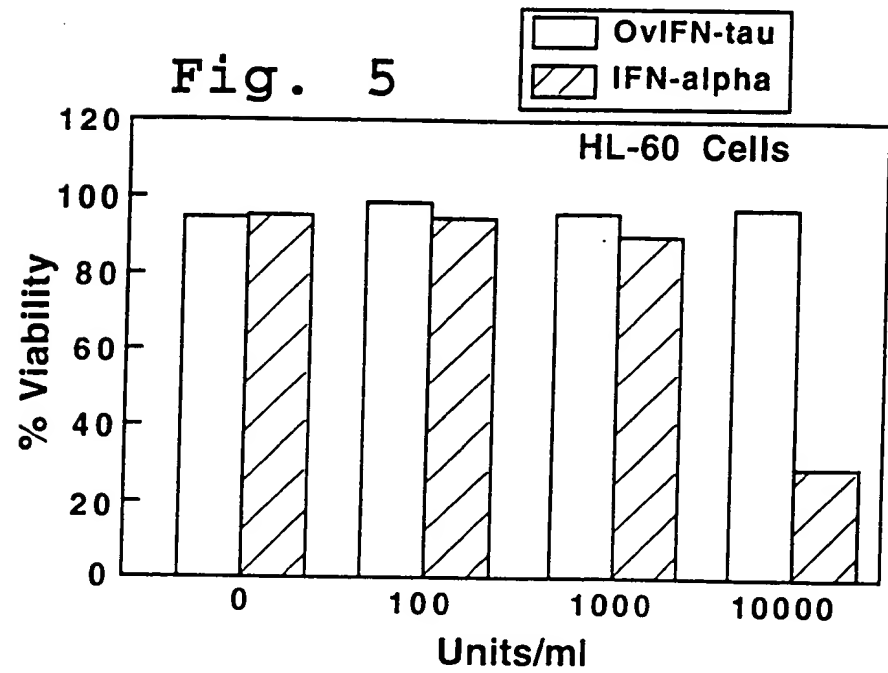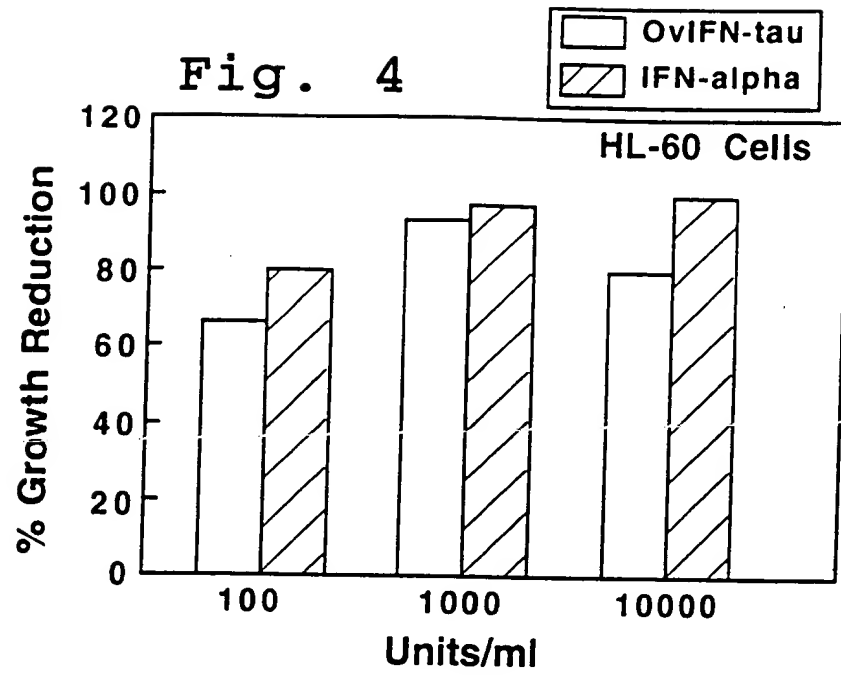
**Fig. 3**

Fig. 4

OvIFN-tau
IFN-alpha

HL-60 Cells

% Growth Reduction

Units/ml



Fig. 5

OvIFN-tau
IFN-alpha

HL-60 Cells

% Viability

Units/ml

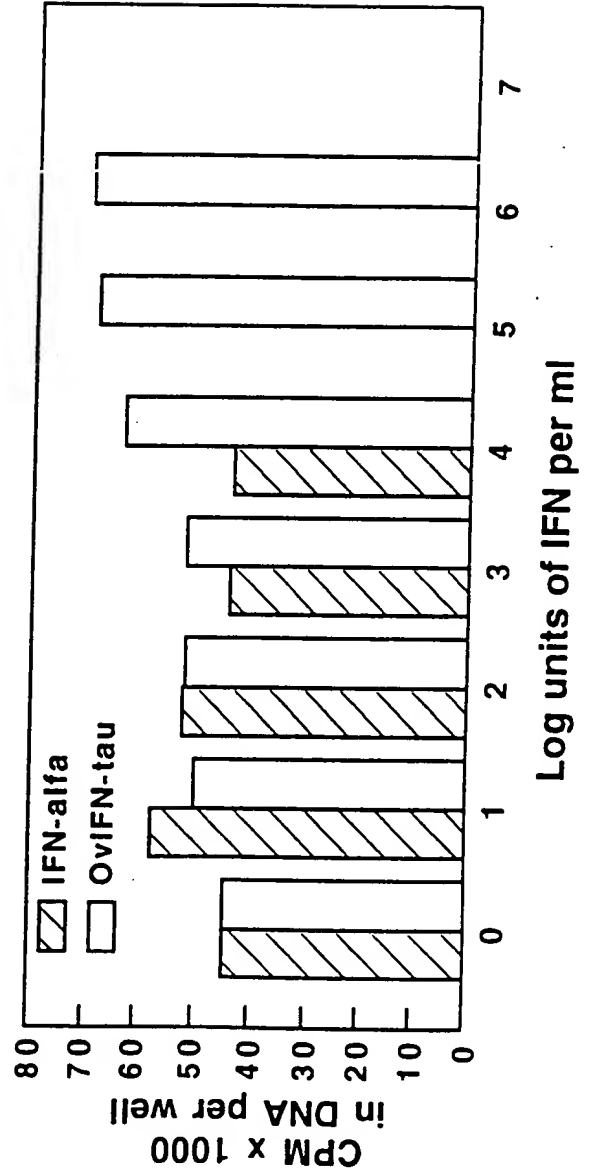| Peptides | MW | HI* | Sequence |
|---|---|---|---|
| IFNt(1-37) (SEQ ID NO:5) | 4465 | -0.78 | CYSLRKLMLDARENLKLLDRMNRLSPHSCLQDRKDFG |
| IFNt(34-64) (SEQ ID NO:6) | 3610 | -0.72 | KDFGLPQEMVEGDQLQKDQAFPVLYEMLQQS |
| IFNt(62-92) (SEQ ID NO:7) | 3586 | -0.53 | QQSFNLFYTEHSSAAWDTTLLEQLCTGLQQQ |
| IFNt(90-122) (SEQ ID NO:8) | 3712 | -0.86 | QQQLDHLDTCRGQVMGEEDSELGNMDPIVTVKK |
| IFNt(119-150) (SEQ ID NO:9) | 3948 | -0.56 | TVKKYFQGIYDYLQEKGYSDCAWEIVRVEMMR |
| IFNt(139-172) (SEQ ID NO:10) | 3818 | -0.11 | CAWEIVRVEMMRALTVSTTLQKRLTKMGGDLNSP |

*Hydropathic Index

Fig. 6



Fig. 8

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```
1                                                                        80
CTGAGATGGGATCAGAGAACCTACCTGAAGGTTCCCCCTGACCCCATCTCAGCCAGCCCAGCAGCAGCCGCATCTTCCCC

81                                                                      140
  ATG GCC TTC GTG CTC TCT CTA CTG ATG GCC CTG GTG CTG GTC AGC TAT GGC CCA GGA GGA
  S1                                                                     S20
  Met Ala Phe Val Leu Ser Leu Leu Met Ala Leu Val Leu Val Ser Tyr Gly Pro Gly Gly
141                                                                     200
  TCT CTG GGT TGT TAC CTA TCT CGG AAA CTC ATG CTG GAT GCC AGG GAG AAC CTC AAG CTC
  S21          1                                                        17
  Ser Leu Gly Cys Tyr Leu Ser Arg Lys Leu Met Leu Asp Ala Arg Glu Asn Leu Lys Leu
201                                                                     260
  CTG GAC CGA ATG AAC AGA CTC TCC CCT CAT TCC TGT CTG CAG GAC AGA AAA GAC TTT GGT
  18                                                                     37
  Leu Asp Arg Met Asn Arg Leu Ser Pro His Ser Cys Leu Gln Asp Arg Lys Asp Phe Gly
261                                                                     320
  CTT CCC CAG GAG ATG GTG GAG GGC GAC CAG CTC CAG AAG GAC CAG GCC TTC CCT GTG CTC
  38                                                                     57
  Leu Pro Gln Glu Met Val Glu Gly Asp Gln Leu Gln Lys Asp Gln Ala Phe Pro Val Leu
321                                                                     380
  TAC GAG ATG CTC CAG CAG AGC TTC AAC CTC TTC TAC ACA GAG CAC TCC TCT GCT GCC TGG
  58                                                                     77
  Tyr Glu Met Leu Gln Gln Ser Phe Asn Leu Phe Tyr Thr Glu His Ser Ser Ala Ala Trp
381                                                                     440
  GAC ACC ACC CTC CTG GAG CAG CTC TGC ACT GGA CTC CAA CAG CAG CTG GAC CAC CTG GAC
  78                                                                     97
  Asp Thr Thr Leu Leu Glu Gln Leu Cys Thr Gly Leu Gln Gln Gln Leu Asp His Leu Asp
441                                                                     500
  ACC TGC AGG GGT CAA GTG ATG GGA GAG GAA GAC TCT GAA CTG GGT AAC ATG GAC CCC ATT
  98                                                                     117
  Thr Cys Arg Gly Gln Val Met Gly Glu Glu Asp Ser Glu Leu Gly Asn Met Asp Pro Ile
501                                                                     560
  GTG ACC GTG AAG AAG TAC TTC CAG GGC ATC TAT GAC TAC CTG CAA GAG AAG GGA TAC AGC
  118                                                                    137
  Val Thr Val Lys Lys Tyr Phe Gln Gly Ile Tyr Asp Tyr Leu Gln Glu Lys Gly Tyr Ser
561                                                                     620
  GAC TGC GCC TGG GAA ATC GTC AGA GTC GAG ATG ATG AGA GCC CTC ACT GTA TCA ACC ACC
  138                                                                    157
  Asp Cys Ala Trp Glu Ile Val Arg Val Glu Met Met Arg Ala Leu Thr Val Ser Thr Thr
621                                                        666
  TTG CAA AAA AGG TTA ACA AAG ATG GGT GGA GAT CTG AAC TCA CCT TGA TGACTCTTGCCGACTA
  158                                                       172
  Leu Gln Lys Arg Leu Thr Lys Met Gly Gly Asp Leu Asn Ser Pro
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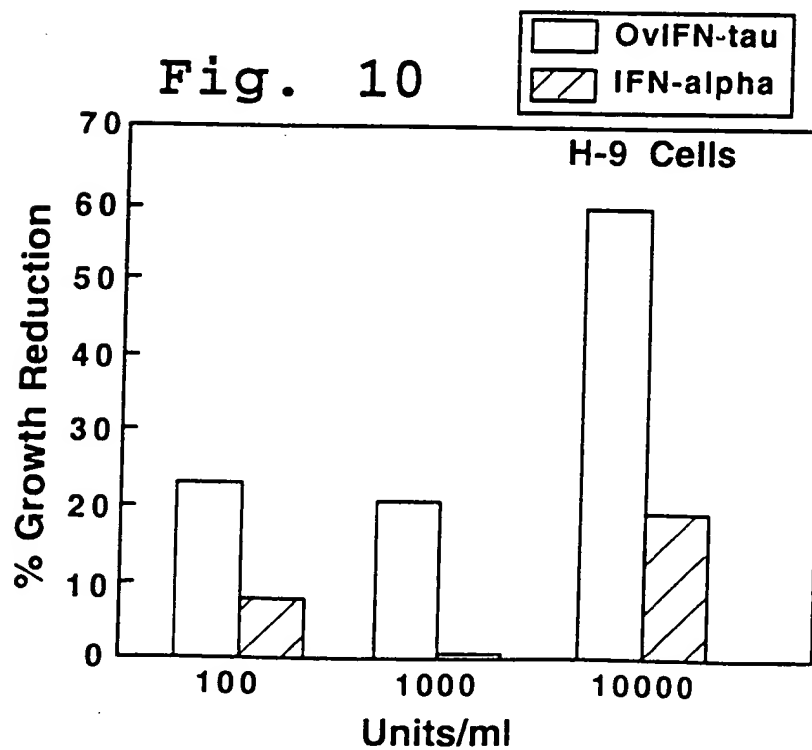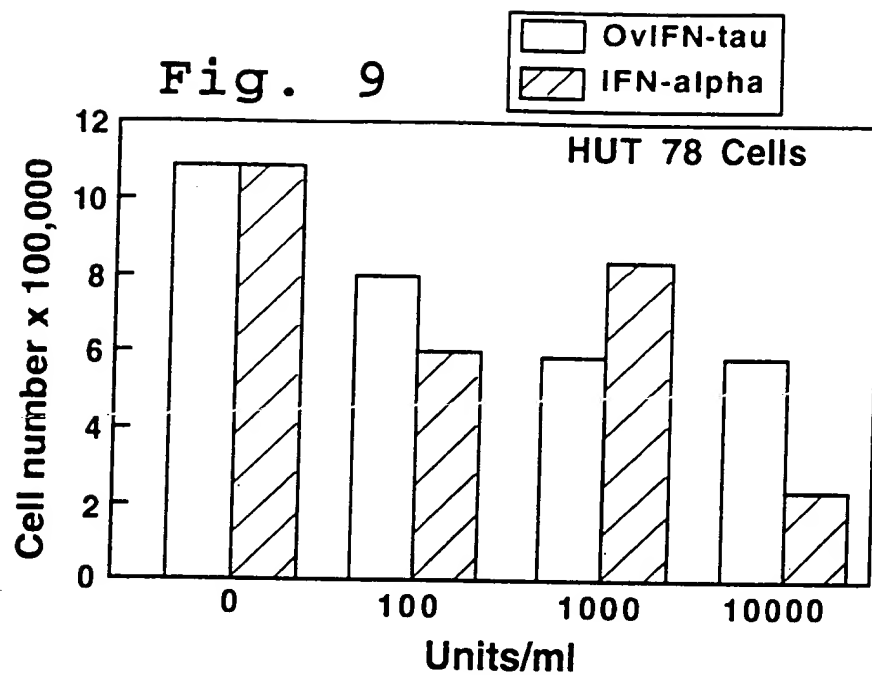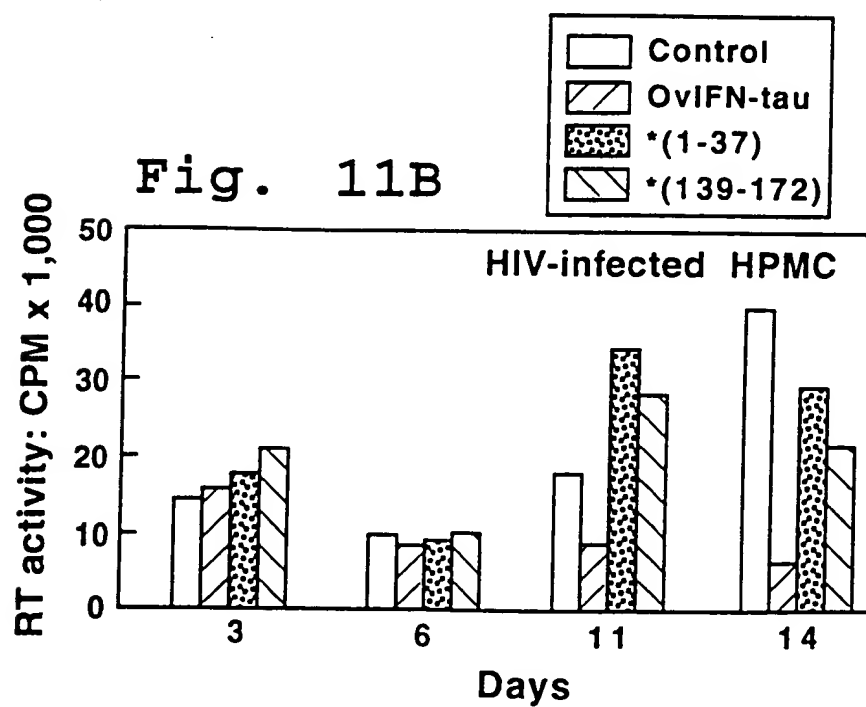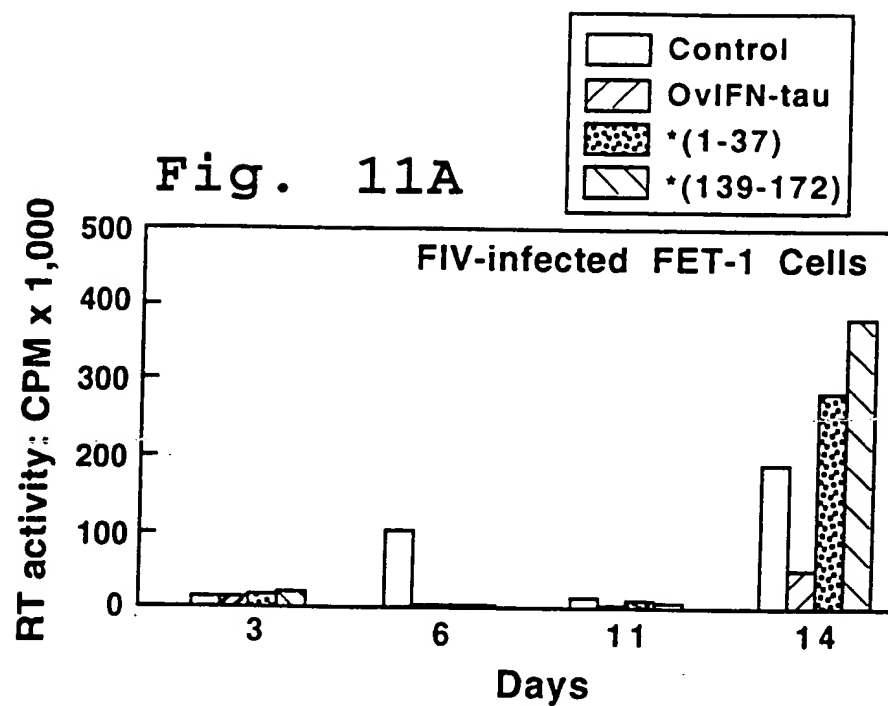
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                                                                        844
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                                                                        924
AATGATGCCTTCCCTGATGTTATCTGTTGCTGATTTATTTATACCTTCTAGCATTTAACATACTTAAAATATTAGGAAAT

                                     972
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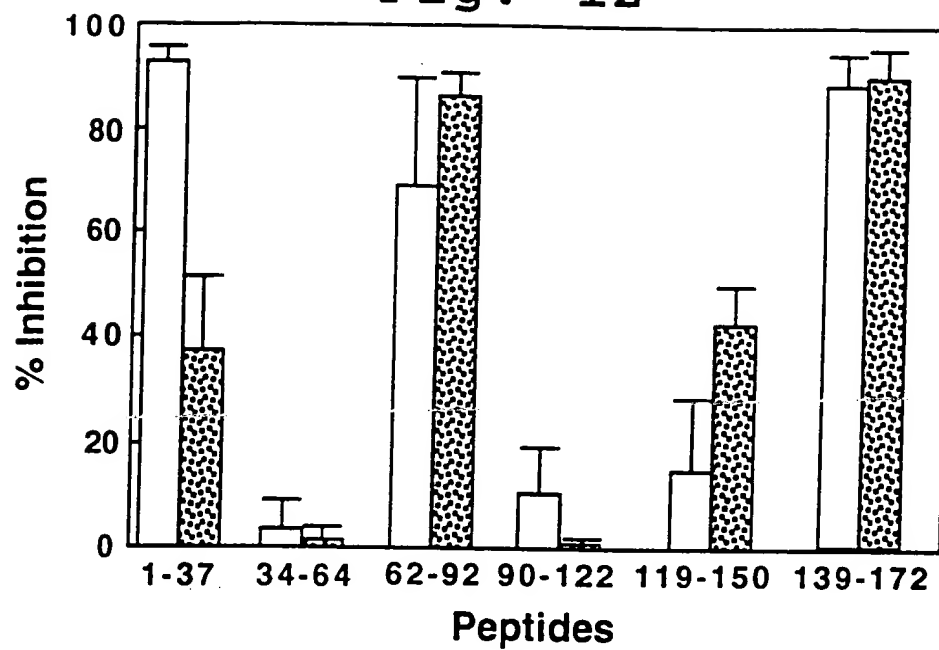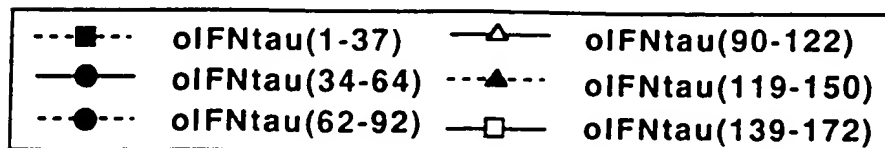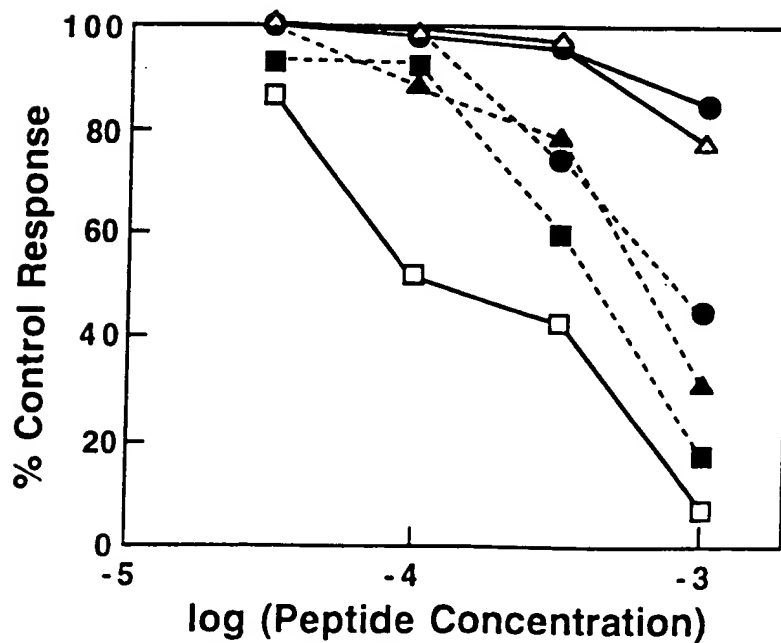
# Fig. 7

Fig.  9

HUT 78 Cells

Legend: OvIFN-tau, IFN-alpha

Cell number x 100,000

Units/ml

Fig.  10

H-9 Cells

Legend: OvIFN-tau, IFN-alpha

% Growth Reduction

Units/ml

## Fig. 11A

| | |
|---|---|
| ☐ | Control |
| ▨ | OvIFN-tau |
| ▨ | *(1-37) |
| ▨ | *(139-172) |

**FIV-infected FET-1 Cells**

RT activity: CPM x 1,000

Days

## Fig. 11B

| | |
|---|---|
| ☐ | Control |
| ▨ | OvIFN-tau |
| ▨ | *(1-37) |
| ▨ | *(139-172) |

**HIV-infected HPMC**

RT activity: CPM x 1,000

Days

## Fig. 12



## Fig. 13

# Fig.  14



Legend:
- - -■- - -  oIFNtau(1-37)     —△—  oIFNtau(90-122)
—●—  oIFNtau(34-64)    - - -▲- - -  oIFNtau(119-150)
- - -●- - -  oIFNtau(62-92)    —□—  oIFNtau(139-172)



# Fig.  15

# Fig. 16



| | |
| --- | --- |
| ---■--- oIFNtau(1-37) | —△— oIFNtau(90-122) |
| —●— oIFNtau(34-64) | ---▲--- oIFNtau(119-150) |
| ---●--- oIFNtau(62-92) | —□— oIFNtau(139-172) |



# Fig. 17

Fig. 18